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ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women

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ABSTRACT

Objectives A simple system for visual inspection with acetic acid (VIA) assessment, named ABCD criteria, has been developed to increase accuracy for triaging of high-risk human papillomavirus (HPV)-positive women. The present study aimed to determine the accuracy of ABCD criteria for the detection of histologically confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in HPV-positive women living in a low-resource setting.

Design Prospective study of diagnostic accuracy

Setting Cervical cancer screening program based on a 3T-Approach (Test, Triage, and Treat) in the Health District of Dschang, West Cameroon.

Participants Asymptomatic non-pregnant women aged 30-49 years were eligible to participate. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. A total of 1980 women were recruited (median age, 40 years; interquartile range, 35–45 years), of whom 361 (18·4%) were HPV-positive and 340 (94·2%) completed the trial.

Interventions HPV-positive women underwent a pelvic examination for visual assessment of the cervix according to ABCD criteria. The criteria comprised A for Acetowhiteness, B for Bleeding, C for Colouring, and D for Diameter. The ABCD criteria results were codified as positive or negative and compared with histological analysis findings (reference standards).

Primary and secondary outcome measures Diagnostic performance of ABCD criteria for CIN2+, defined as sensitivity, specificity, negative and positive predictive values.

Results ABCD criteria had a sensitivity of 77·5% (95% CI, 61·3%–88·2%), specificity of 42·0% (95% CI, 36·5%–47·7%), positive predictive value of 15·1% (95% CI, 10·8%–20·8%), and negative predictive value of 93.3% (95% CI, 87·6%–96·5%) for detection of CIN2+ lesions. Most (86·7%) of the ABCD-positive women were treated on the same day.

Conclusions ABCD criteria can be used in the context of a single-visit approach and may be the preferred triage method for management of HPV-positive women in a low-income context.

Trial registration The trial was registered under ClinicalTrials.gov (number NCT03757299).

Key words: cervical cancer screening, low- and middle-income countries, visual inspection with acetic acid (VIA), visual inspection with Lugol's iodine (VILI), human papillomavirus, triage

Strengths and limitations of this study

- Using ABCD criteria for D-VIA interpretation is a simple test with binary results (positive or negative) that are immediately available, allowing initiation of therapy without delay.

- Because all HPV-positive women underwent biopsy and cervical brushing regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity.
- A limitation of the study was its setting in a single centre in a district hospital in West Cameroon with five clinicians administering all screening and treatment procedures.

INTRODUCTION

More than 80% of cervical cancer (CC) deaths occur in low- and middle-income countries (LMICs), mainly due to lack of prevention.[1] Cytology-based CC screening programs and more recent HPV-based programs have been successfully implemented in high-income countries and have been associated with important reductions in deaths from CC.[2] However, these strategies have not been implemented in LMICs, predominantly because of financial and logistical limitations. Alternative methods such as visual inspection of the cervix after application of acetic acid (VIA) are considered suitable for use in LMICs.[3,4]

The World Health Organization (WHO) recommendations for screening in resource-limited settings include a strategy of HPV-screening followed by VIA and treatment, or a strategy of HPV-screening and treatment.[3] Although no recommendations are given for the approach that should be prioritized, sub-Saharan Africa has a high HPV prevalence rate of 15%–30% and most HPV-positive women have no lesions.[3,7,8] In this context, HPV testing followed by immediate treatment can represent significant overtreatment in women with an HPV-positive test, which by itself may not confer a high risk of cervical intraepithelial neoplasia grade 2 or worse (CIN2+).[5–9] In sub-Saharan Africa, the prevalence of CIN2+ was reported to be 2%–4% in women aged 30–49 years and 7%–11% in an HPV-positive population with a low HIV

prevalence rate (<10%).[7–9] A triage system is only a valid option if it can conserve the high sensitivity of the HPV test for identifying CIN2+ disease.

Triage by VIA and/or visual inspection with Lugol's iodine (VILI) requires accurate criteria to decide whether or not the findings are positive, which are generally based on the International Agency for Research against Cancer (IARC) manual.[10] However, in this setting, VIA triage in HPV-positive populations appears to be associated with an important loss of sensitivity, suggesting that triage by VIA using traditional criteria may not be of benefit.[7–10] Previous studies using histology as reference standard and having excluded verification bias had sensitivities ranging from 25.0% to 45.5%.[7,9,11] In a pilot study having used relaxed criteria for VIA interpretation in HPV-positive women, sensitivity increased to 80%.[8] Interpreting VIA with naked eye alone is subjective and is highly variable between health care providers.[12-14] This issue may be improved with continuous supervision and medical education thanks to the use of digital VIA and VILI (D-VIA/D-VILI). This includes acquisition of cervical images, native and after VIA and VILI application, through a camera or smartphone. These technologies provide an alternative to colposcopy in the context of LMICs and may constitute an important step in the improvement of VIA/VILI interpretation.[15–17] Although the image quality is probably lower than that with high-resolution colposcopy, there are significant benefits for healthcare providers, because they can move through and

compare the native, VIA, and VILI images, and can also magnify suspicious lesions, before deciding whether treatment is needed.[15,16]

To improve VIA/D-VIA interpretation as a triage test in HPV-positive populations, we introduced a set of criteria, termed ABCD criteria. These criteria constitute a simple structure that may contribute to preventing CC in an LMIC context. The aim of the present study was to provide a rationale for the ABCD criteria and determine their performance in identifying histology-proven CIN2+.

METHODS

Study design – This prospective study was carried out between September 2018 and March 2020 in the health district of Dschang (West Cameroon). Asymptomatic non-pregnant women aged 30-49 years were eligible to participate in the study on a voluntary basis and were included in a consecutive manner upon presentation to the screening site. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. The study was conducted within a larger trial aiming to recruit 6,000 women in a 5-year screening program.[17] At the baseline visit, after obtaining written informed consent and providing guidance to participants on the procedure for vaginal self-sampling, participants undertook an HPV self-test (Self-HPV) that was subsequently analyzed by a point-of-care assay

(GeneXpert®) on the same day. HPV-negative women were reassured and advised to repeat the test in 5 years, while HPV-positive women were invited to undergo visual triage and thermal ablation or large loop excision of the transformation zone (LLETZ) if needed.

ABCD criteria (Figure 1) – The ABCD criteria were chosen from a synthesis of published results as well as our own experience in VIA and VILI interpretation.[3,10,18–22] We considered acetowhiteness as the most important predictor for CIN and noted that Lugol's iodine can be used to identify thin acetowhite lesions not seen on the initial VIA assessment (Figure 1). Similar to the IARC criteria, the pathological area should be located within or in contact with the transformation zone (TZ). The ABCD criteria are codified as positive (present) or negative (absent). To be considered ABCD-positive, at least one of the following conditions needs to be fulfilled: presence of criteria A (acetowhiteness) and D (diameter) combined, or criterion B (bleeding) with or without presence of A, C (colouring) or D.

ABCD criteria were independently evaluated by one of three trained midwives and supervised by two experienced Cameroonian gynaecologists. ABCD criteria interpretations were performed first in real-time during VIA/VILI, and on smartphone images, before deciding whether or not to perform treatment. A set of three images (native, acetic acid, Lugol's iodine) were obtained on a Galaxy S5 smartphone (Samsung, Seoul, South Korea).

Diagnosis and treatment were based on combined results of VIA/VILI and smartphone-

enhanced D-VIA, using aids such as zooming in on lesions and performing comparisons between the native, VIA, and VILI images. A positive ABCD result by either one of VIA/VILI or D-VIA/D-VILI warranted treatment.

Eligiblity criteria for thermal ablation were women being positive for ABCD criteria.

Indications for referral to a gynecologist to determine treatment modalities were (i) lesions extending into the endocervix which could not be covered by the probe tip, (ii) suspicion of carcinoma, in-situ adenocarcinoma or invasive adenocarcinoma, (iii) presence of bleeding and (iv) presence of acetowhite lesions covering more than 75% of the ectocervix. Our management of HPV-positive women with a TZ type 3 was as follows: (i) those having no lesion on visual assessment were offered follow-up, (ii) those having a lesion which could be covered by thermal ablation tips were treated, and (iii) those with an endocervical lesion which could not be fully covered by the probe were referred for LLETZ. Cervical liquid-based cytology, biopsy at the TZ and endocervical curettage (ECC) were performed on all HPV-positive women prior to treatment.

Cytology – Cervical liquid-based cytology was performed using the SurePath (September 2018 to July 2019) and ThinPrep (July 2019 to March 2020) techniques. All vials were analyzed in Switzerland (CytoPath, Unilabs, Geneva, and University Hospital of Geneva). The slides were independently read by qualified cytotechnologists and classified according to

the Bethesda classification system: negative for intraepithelial lesion or malignancy (NILM), inflammatory atypical squamous cells of undetermined significance (ASC-US), inflammatory atypical squamous cells that cannot exclude HSIL (ASC-H), atypical glandular cells with low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and invasive cancer.

Histology findings (reference standard) – Cervical biopsies were performed using biopsy forceps, and ECC was carried out with an endocervical brush. Cervical biopsies were performed at 6 o'clock in the TZ when ABCD criteria were negative. If ABCD criteria were positive, one or more biopsies were performed at the most suspicious areas. All samples were stored in formalin. Biopsy slides and ECC samples were read by two experienced gynaecologic pathologists who were blinded to the screening test results and ABCD criteria findings. The histological results were classified as normal, CIN1, CIN2, CIN3, adenocarcinoma *in situ* (AIS), invasive carcinoma, or adenocarcinoma. The cut-off for a pathological result was set at CIN2+. When histological results varied within the samples of one participant, only the worst result was considered as the reference standard.

Patient and public involvement – Preferences of and experience with former patients of a preliminary research study on cervical cancer screening in Dschang, Cameroon, were considered in the design and conduction of this study. During the study, focus groups were

organized with members of the community (women and men), health care workers and community health workers, to explore barriers to cervical cancer screening and further improve the program and recruitment strategy. Patients are also involved at their arrival at the screening center where they are offered a one-hour information session on cervical cancer and sexual health by trained midwives. Furthermore, the public is kept informed about the progress of our research through the publication of yearly newsletters disseminated among health workers and the general community.

Statistical analysis – Initially, we planned a sample of 6,000 women. However, the COVID-19 pandemic and public health measures to control the virus have impacted on-site clinical activity since mid-March 2020. In this context, we decided to consider an interim analysis to the trial of the primary endpoints which included performance of the ABCD criteria. Descriptive statistics were used to analyse the baseline characteristics of the study population. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) plus their 95% confidence intervals (95% CIs) were calculated. Student's *t*-test, Mann–Whitney test, or Pearson's chi-square test were used, where appropriate, to identify sociodemographic and reproductive characteristics of the patients that could differ between ABCD criteria results. A P-value of <0·05 was considered statistically significant. An exploratory analysis was performed to assess the relationships between each independent

variable and the correct prediction of the ABCD criteria. This correct prediction score was equal to 1 when ABCD criteria were positive and there was a CIN2+ on histology or if the ABCD criteria were negative and histology was also negative. All other incorrect predictions were assigned the value 0. Univariate and multivariate logistic regression analyses were carried out to identify predictors of a correct ABCD criteria score according to histology. Participants with missing or indeterminate results for ABCD criteria or histopathology were excluded from the analysis. Odds ratios (ORs) were adjusted for potential confounders, such as age, marital status, number of lifetime sexual partners, age at first sexual intercourse, age at first delivery, parity, HIV status, and type of TZ, and 95% CIs were calculated. All data analyses were conducted using Stata Statistical software Release 13 (StataCorp LP, College Station, TX).

Ethical considerations – The study obtained approval from the Cantonal Ethics Board of Geneva, Switzerland (Commission cantonale d'éthique de la recherche [CCER], No. 2017-0110) and the Cameroonian National Ethics Committee for Human Health Research (No. 2018/07/1083/CE/CNERSH/SP). The trial was registered under ClinicalTrials.gov (number NCT03757299). The full study protocol can be provided upon request to the first author.

RESULTS

A total of 1980 women aged 30–49 years were enrolled (median age: 41 years; interquartile range [IQR], 36–50 years). Overall, 1964 women performed Self-HPV, of whom 361 (18·5%) had an HPV-positive test and underwent pelvic examination, three were excluded from the results analysis for lack of ABCD criteria assessment, and 340 (94·2%) had interpretable histology findings and constituted the study population (**Figure 2**). **Table 1** provides details of the baseline sociodemographic, reproductive, and clinical characteristics of the participants. Median age at first sexual intercourse was 18 years (IQR, 16–19 years) and median number of sexual lifetime partners was 3 (IQR, 2–5).

Table 1: Baseline sociodemographic, reproductive health, and clinical characteristics according to ABCD criteria (N=358)*

Variable	ABCD criteria-negative	ABCD criteria-positive	Total	P-value
Participants recruited, n (%)	140 (39.1)	218 (60.9)	358	
Age (years), median (IQR)	41 (35–45)	40 (34–45)	40 (34–45)	0.4464
Marital status, n (%)				0.8910
Single	15 (10.7)	20 (9.2)	35 (9.8)	
With partner	109 (77.9)	173 (79.3)	282 (78.8)	
Divorced/widowed	16 (11.4)	25 (11.5)	41 (11.4)	
Education, n (%)				0.3900
Unschooled	1 (0.7)	5 (2.3)	6 (1.7)	
Primary education	37 (26.4)	66 (30.3)	103 (28.8)	
Secondary education	67 (47.9)	105 (48.2)	172 (48.0)	
Tertiary education	35 (25.0)	42 (19.2)	77 (21.5)	
Employment status, n (%)				0.1750
Employed	50 (35.7)	57 (26.2)	107 (29.9)	
Independent	39 (27.9)	56 (25.7)	95 (26.5)	
Housewife	23 (16.4)	41 (18.8)	64 (17.9)	
Unemployed	7 (5.0)	12 (5.5)	19 (5.3)	
Farmer	21 (15.0)	52 (23.8)	73 (20.4)	
Age at menarche (years), mean ± SD	14.7±1.8	14.7±1.9	14.7±1.8	0.8914
Age at first intercourse, median (IQR)	17 (16–19)	18 (16–20)	18 (16–19)	0.2390
Number of sexual partners, median	4 (3–6)	3 (2–5)	3 (2–5)	**0.0008**
Contraception, n (%)				0.5950
None	93 (66.9)	142 (65.5)	235 (66.0)	
Condom	18 (13.0)	25 (11.5)	43 (12.1)	

Hormonal pill	1 (0.7)	7 (3.2)	8 (2.3)	
DIU/ implant/ injection	25 (18.0)	41 (18.9)	66 (18.5)	
Other	2 (1.4)	2 (0.9)	4 (1.1)	
HIV status, n (%)				0.9420
Negative	128 (92.7)	198 (93.0)	326 (92.9)	
Positive	10 (7.3)	15 (7.0)	25 (7.1)	
Age at first delivery (years), mean ± SD	21.4±3.7	21.4±2.5	21.4±3.8	0.9137
Parity, n (%)				**0.0080**
Nulliparous	11 (7.9)	3 (1.4)	14 (3.9)	
1–4	66 (47.1)	108 (49.5)	174 (48.6)	
>4	63 (45.0)	107 (49.1)	170 (47.5)	
Transformation zone, n (%)				**<0.0001**
TZ1	76 (57.1)	150 (73.5)	226 (67.1)	
TZ2	26 (19.6)	45 (22.1)	71 (21.1)	
TZ3	31 (23.3)	9 (4.4)	40 (11.8)	
HPV testing results, n (%)				
HPV-16	11 (7.9)	23 (10.6)	34 (9.5)	0.3890
HPV-18/45	22 (15.8)	31 (14.2)	53 (14.9)	0.6770
Other HPV	114 (82.0)	186 (85.3)	300 (84.0)	0.4060
Cytology, n (%) (Total= 343)				0.0990
Normal	108 (82.5)	161 (75.9)	269 (78.4)	
ASC-US	7 (5.3)	10 (4.7)	17 (5.0)	
LSIL	10 (7.6)	15 (7.1)	25 (7.3)	
HSIL	4 (3.1)	21 (9.9)	25 (7.3)	
ASC-H	0	4 (1.9)	4 (1.2)	
Cancer	2 (1.5)	1 (0.5)	3 (0.8)	
Histology, n (%) (Total=340)				**0.0040**
Normal	108 (80.0)	129 (62.9)	237 (69.7)	
CIN1	18 (13.3)	45 (21.9)	63 (18.5)	
CIN2	1 (0.7)	12 (5.9)	13 (3.8)	
CIN3	6 (4.4)	18 (8.8)	24 (7.1)	
Invasive cancer	2 (1.5)	1 (0.5)	3 (0.9)	

Abbreviations: SD = standard deviation; IQR = interquartile range; CIN1 = cervical intraepithelial neoplasia grade 1; CIN2 = cervical intraepithelial neoplasia grade 2; CIN3 = cervical intraepithelial neoplasia grade 3; HIV = human immunodeficiency virus; HPV = human papillomavirus.

*Data from the 358 participants may be missing for some variables.

Thirty-four (9·5%) samples were positive for HPV-16, 53 (14·9%) for HPV-18/45 and 300 (84·0%) for other HPV types. Overall, 218 (60·9%) participants were classified as ABCD criteria-positive. All patients positive for ABCD were treated with thermal ablation with the exception of one patient who underwent LLETZ and one patient suspicious of cancer who was biopsied and referred for multimodal therapy. Thermal ablation was provided on the same day as HPV screening in 86.7% of cases. Reasons for delaying treatment included

referral for further evaluation, technical issues, bleeding at the time of screening, or choice of the patients themselves. No serious adverse event occurred as a result of the screening procedure.

Among all 358 women with HPV-positive results, 343 samples with valid cytological results and 340 samples with valid histological results were obtained. Of the 343 valid cytological results, 21·6% had abnormal cytology (ASC-US+). Four patients had ASC-H, 25 had HSIL, and three had cytology suggesting cancer. All three cancers identified by cytology were confirmed by histology. Of the 340 valid histological results, 63 (18·5%) CIN1 were identified, 13 (3·8%) CIN2, 24 (7·1%) CIN3, and 3 (0·9%) invasive cancers. The prevalence of CIN2+ and CIN3+ was 11·8% and 7·9%, respectively. Details for the disease prevalences are also shown in **Table 1**.

Table 2 shows demographic and pathological characteristics associated with a correct prediction of the ABCD criteria.

Table 2: Demographic and pathological characteristics associated with a correct prediction of the ABCD criteria (N=340)*

Variable	Total	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)**	P-value
Age (years), n (%)					
30–40	186 (54.7)	1.00 (Reference)		1.00 (Reference)	
41–50	154 (45.3)	1.39 (0.90–2.14)	0.133	1.51 (0.87–2.60)	0.140
Marital status, n (%)					
Single	34 (10.0)	1.00 (Reference)		1.00 (Reference)	
With partner	265 (77.9)	1.15 (0.56–2.36)	0.706	1.07 (0.43–2.63)	0.887
Divorced/widowed	41 (12.1)	0.81 (0.32–2.04)	0.656	0.63 (0.19–2.04)	0.442
Education, n (%)					
Unschooled/primary education	101 (29.7)	1.00 (Reference)		1.00 (Reference)	
Secondary/tertiary education	239 (70.3)	1.04 (0.65–1.65)	0.879	0.92 (0.47–1.82)	0.818
Employment status, n (%)					

Employed	104 (30.6)	1.00 (Reference)		1.00 (Reference)	
Independent	93 (27.3)	0.90 (0.51–1.57)	0.706	0.73 (0.38–1.43)	0.363
Housewife	58 (17.1)	0.81 (0.43–1.55)	0.528	0.74 (0.34–1.63)	0.461
Unemployed	19 (5.6)	0.72 (0.27–1.95)	0.528	0.89 (0.27–2.91)	0.852
Farmer	66 (19.4)	0.69 (0.37–1.29)	0.248	**0.41 (0.18–0.95)**	**0.037**
Age at first intercourse (years), n (%)					
≤17	154 (45.6)	1.00 (Reference)		1.00 (Reference)	
≥18	184 (54.4)	0.70 (0.46–1.08)	0.106	0.75 (0.43–1.31)	0.315
Number of sexual partners†, median	**3 (2–5)**	**1.08 (1.01–1.16)**	**0.031**	1.06 (0.97–1.17)	0.176
1–2, n (%)	98 (28.8)	1.00 (Reference)		1.00 (Reference)	
3–5, n (%)	177 (52.1)	1.39 (0.84-2.30)	0.195	1.22 (0.67-2.22)	0.506
>5, n (%)	**65 (19.1)**	**1.96 (1.04-3.70)**	**0.038**	1.53 (0.70–3.38)	0.284
Contraception, n (%)					
No	225 (66.6)	1.00 (Reference)		1.00 (Reference)	
Yes	113 (33.4)	0.84 (0.54–1.33)	0.466	0.92 (0.54–1.85)	0.769
HIV status, n (%)					
Negative	309 (92.8)	1.00 (Reference)		1.00 (Reference)	
Positive	24 (7.2)	1.21 (0.53–2.77)	0.657	0.95 (0.36–2.53)	0.589
Age at first delivery (years), n (%)					
≤20	157 (47.7)	1.00 (Reference)		1.00 (Reference)	
≥21	172 (52.3)	0.70 (0.45–1.08)	0.102	0.60 (0.34–1.07)	0.085
Parity, n (%)					
Nulliparous	14 (4.1)	1.00 (Reference)		1.00 (Reference)	
1–4	**165 (48.5)**	**0.21 (0.06–0.79)**	**0.020**	0.26 (0.02-2.91)	0.274
>4	**161 (47.4)**	**0.23 (0.06–0.86)**	**0.029**	0.28 (0.02-3.22)	0.307
Transformation zone, n (%)					
TZ1	210 (65.8)	1.00 (Reference)		1.00 (Reference)	
TZ2	70 (22.0)	1.17 (0.68–2.02)	0.575	1.24 (0.67-2.26)	0.492
TZ3	**39 (12.2)**	**6.72 (2.84–15.93)**	**<0.0001**	**6.47 (2.59-16.21)**	**<0.0001**
HPV testing results, n (%)					
Other HPV (without co-infection)	264 (77.9)	1.00 (Reference)		1.00 (Reference)	
HPV-16/18/45	75 (22.1)	1.19 (0.70–1.98)	0.514	1.18 (0.64–2.17)	0.605
Cytology, n (%)					
High-grade+***	**29 (8.9)**	**2.47 (1.11–5.49)**	**0.027**	**3.37 (1.35–8.44)**	**0.009**
Histology, n (%)					
CIN2+	**40 (11.8)**	**4.76 (2.18–**	**<0.0001**	**6.05 (2.47–14.77)**	**<0.000**

Abbreviations: 95% CI = 95% confidence interval; CIN2+ = cervical intraepithelial neoplasia grade 2 or worse.

*Data from the 340 participants may be missing for some variables.

†ORs for continuous variables indicate the change in odds for an increase of one standard deviation.

**Adjusted for age, marital status, age at first intercourse, number of lifetime sexual partners, age at first delivery, parity, HIV status, and type of transformation zone.

***High-grade lesions include ASC-H, HSIL, AIS, and cancer.

Bold values are statistically significant.

ABCD criteria were more likely to be correct in the presence of TZ type 3 (aOR = 6.47; 95% CI, 2.59–16.21; P<0·001), high-grade lesions on cytology (aOR = 3.37; 95% CI, 1.35–8.44; P<0·009) and a CIN2+ on histology (aOR = 6.05; 95% CI, 2.47–14.77; P<0·001). Overall, a

correct prediction of the ABCD criteria was not impacted by the multiple sociodemographic characteristics of the population in the multivariate analysis.

Performance of ABCD and cytology for detection of high-grade cervical lesions (CIN2+ and CIN3+) is shown in **Table 3**.

Table 3: Diagnostic accuracy of ABCD criteria, cytology, and HPV for detection of CIN2+ and CIN3+

	CIN2+ (N=40, 11.8%)			
Variable	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
ABCD criteria-positive	77.5 (61.3–88.2)	42.0 (36.5–47.7)	15.1 (10.8–20.8)	93.3 (87.6–96.5)
Cytology ASC-US+	80.0 (64.0–89.9)	87.5 (83.1–90.7)	47.1 (35.3–59.2)	96.9 (93.9–98.5)
Cytology LSIL+	70.0 (53.5–82.6)	91.3 (87.4–94.1)	52.8 (39.1–66.2)	95.6 (92.4–97.5)
Cytology HSIL+	62.5 (46.1–76.5)	98.6 (96.3–99.5)	86.2 (67.0–95.1)	95.0 (91.8–97.0)
HPV-16/18/45+	37.5 (23.5–53.9)	79.9 (74.9–84.1)	20.9 (12.3–30.8)	90.5 (86.3–93.5)
	CIN3+ (N=27, 7.9%)			
	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
ABCD criteria-positive	70.4 (49.6–85.2)	40.6 (35.2–46.1)	9.3 (6.0–14.1)	94.1 (88.5–97.0)
Cytology ASC-US+	88.9 (68.9–96.7)	85.4 (80.9–89.0)	35.3 (24.7–47.6)	98.8 (96.4–99.7)
Cytology LSIL+	81.5 (60.9–92.5)	89.7 (85.7–92.7)	41.5 (28.7–55.5)	98.2 (95.7–99.2)
Cytology HSIL+	74.1 (53.2–87.8)	97.0 (94.3–98.4)	68.9 (49.0–83.7)	97.7 (95.2–98.9)
HPV-16/18/45+	44.4 (26.2–64.3)	79.8 (75.0–83.9)	16.0 (9.2–26.4)	94.3 (90.8–96.6)

Abbreviations: CIN2+ = cervical intraepithelial neoplasia grade 2 or worse; CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; Cytology ASC-US+ = ASC-US, LSIL, ASC-H, HSIL, AIS, and cancer; Cytology LSIL+ = LSIL, ASC-H, HSIL, AIS, and cancer; Cytology HSIL+ = ASC-H, HSIL, AIS, and cancer; HPV = human papilloma virus; HPV-16/18/45+ = HPV DNA test positive for HPV-16, HPV-18, and HPV-45; 95% CI = 95% confidence interval; PPV = positive predictive value; NPV = negative predictive value.

ABCD criteria for CIN2+ detection showed a sensitivity of 77·5% (95% CI, 61·3%–88·2%), specificity of 42·0% (95% CI, 36·5%–47·7%), PPV of 15·1% (95% CI, 10·8%–20·8%), and NPV of 93·3% (95% CI, 87·6%–96·5%). Cytology-classified HSIL+ for CIN2+ detection showed lower sensitivity of 62·5% (95% CI, 46·1%–76·5%), but higher specificity of 98·6% (95% CI, 96·3%–99·5%), PPV of 86·2% (95% CI, 67·0%–95·1%), and NPV of 95·0% (95% CI, 91·8%–97·0%). Meanwhile, cytology-classified ASC-US+ showed improved sensitivity of 80·0% (95% CI, 64·0%–89·9%) and specificity of 87·5% (95% CI, 83·1%–90·7%). Screening by HPV 16/18/45 genotyping alone had a much lower sensitivity of 37·5% (95% CI, 23·5–53·9) and a specificity of 79.9% (95% CI 74·9–84·1). ABCD criteria for CIN3+ lesion identification showed a sensitivity of 70·4% (95% CI, 49·6%–85·2%), specificity of 40·6% (95% CI, 35·2%–46·1%), PPV of 9·3% (95% CI, 6·0%–14·1%), and NPV of 94·1% (95% CI, 88·5%–97·0%).

DISCUSSION

The ABCD criteria were established as part of our efforts to improve the performance of visual-based approaches for triage of HPV-positive women. Previous studies conducted in LMICs indicated that traditional VIA criteria were not satisfactory for the detection of CIN2+ lesions, with a trend toward reduced sensitivity compared with HPV testing alone.[7–9] The

challenge for VIA screeners lies in interpreting the wide variability of cervical presentations, in populations where obstetric trauma to the cervix and history of infection are frequent, and in which CIN2+ may be difficult to identify by the naked eye alone.

The most important finding of this study is that the ABCD criteria appeared to be highly sensitive for detection of high-grade lesions in an HPV-positive population. We used both (i) a magnification technique with smartphone digital imaging that allows more detailed examination compared with naked eye alone and (ii) a lower VIA/D-VIA threshold positivity to optimize identification of lesions. The ABCD criteria provided improved VIA sensitivity for triage of HPV-positive women compared to most studies published using a comparable methodology (sensitivities ranging from 25% to 45.5%), and the weakness was the low specificity (42%, with previous specificities ranging from 44% to 98%). [7-9,11,23] This can be explained by the fact that the IARC criteria require extensive VIA changes before being considered positive, thus limiting their sensitivity, while a reduced positivity threshold can contribute to improved sensitivity for CIN2+ detection.[10,20]

The low specificity arises because we considered any whitening to be positive, meaning many benign conditions (metaplasia, inflammation or other benign cervical changes) could produce false-positive results for the ABCD criteria. Criterion C (VILI/D-VILI), though dependent on criteria A and D, may contribute to the high false positive rate by categorizing

benign conditions as ABCD-positive through the identification of iodine-negative areas compatible with thin, transparent or patchy acetowhite lesions on D-VIA. The lack of association between multiple socio-demographic variables and a correct prediction of the ACBD criteria (**Table 2**) supports the generalizability of these criteria to the overall population of women aged 30 to 49 years.

Compared to screening by HPV-16/18/45 genotyping without triage, the sensitivity of the ABCD criteria was much higher, at the cost of a lower specificity. PPV was also slightly lower with triage by ABCD criteria (15·1%) than with HPV genotyping. Overall, 54·4% of normal histology results and 71·4% of CIN1 were considered ABCD criteria positive and consequently underwent unnecessary treatment. Thus, 85% (174 of 205) of women who screened positive were treated unnecessarily. However, when considering all women screened for CC, including HPV-negative, 174 were treated unnecessarily out of 1964 screened by Self-HPV, corresponding to an overall 8·9% overtreatment rate in the total population screened. Despite the low specificity, our 3T-Approach in a single visit may be acceptable in an LMIC context because it reduces cost and loss to follow-up. Furthermore, treatment by thermal ablation has low risks of side effects and morbidity.[24] Therefore, treatment of a significant number of false-positive cases may be considered an acceptable strategy for effective control of CC in an LMIC setting. The second limitation is that the study

was conducted in a single centre in a district hospital in West Cameroon with five clinicians (three midwives and two gynaecologists) administering all screening and treatment procedures.

It should be noted that two out of three cervical cancers were assessed as ABCD-negative on site by the frontline health care providers and did not receive immediate treatment. After reviewing the smartphone images of these two cases off-site, it was determined that criterion B (bleeding) was present in both cases, which should have led to a positive ABCD result and subsequent treatment (**Supplement, Figure S1**).

The strength of ABCD criteria is that they comprise a simple tool that can alert healthcare professionals to the clinical features of CIN2+, and the use of “relaxed IARC criteria” may greatly decrease the risk of missing CIN2+ lesions. Using ABCD criteria for D-VIA interpretation is a simple test with binary results (positive or negative) that are immediately available, allowing initiation of therapy without delay. In our series, 86·7% of participants underwent the 3T-Approach in one day.

Furthermore, because all HPV-positive women underwent biopsy and cervical brushing regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity for ABCD criteria.

In conclusion, ABCD criteria can improve CIN2+ diagnosis in HPV-positive women using VIA and D-VIA. This approach may provide a unique opportunity to improve cervical cancer screening programs in LMICs using a one-visit approach. This strategy may be particularly beneficial because the criteria are easily remembered and easy to use for healthcare providers.

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Competing Interests

All authors declare that they have no competing interests.

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Data access, analysis and responsibility

The principal investigator had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Data used in the study is available upon request to the first author.

Contributors

PP, BK, and PV designed the study protocol, implemented the study, oversaw the data collection, analysed the data, and drafted and revised the paper. AW and RC conducted data analysis, interpreted the data, and revised the draft paper. BK, ET, and JF trained the study staff, assumed the quality control (supervision and mentorship), supported the data collection, interpreted the data, and revised the draft paper. JCT and ES analysed the pathological specimens, interpreted the data, and revised the draft paper.

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Figure 1: ABCD criteria for VIA interpretation in HPV-positive women

Figure 2: Flowchart of participants for the 3T-Approach in Cameroon

Figure 1: ABCD criteria for VIA interpretation in HPV-positive women

Criterion A – **A**cetowhite area touching the transformation zone (absent on the native view and apparent after acetic acid application) is considered positive.

Criterion B – **B**leeding without touching or after lightly touching (with a swab or speculum) the cervix is considered positive.

Criterion C (optional) – **C**olouring with VILI contributes to confirmation or identification of a faint acetowhite lesion.

Criterion D – Diameter of >5 mm (about the size of a pencil eraser) in an acetowhite area is considered positive.

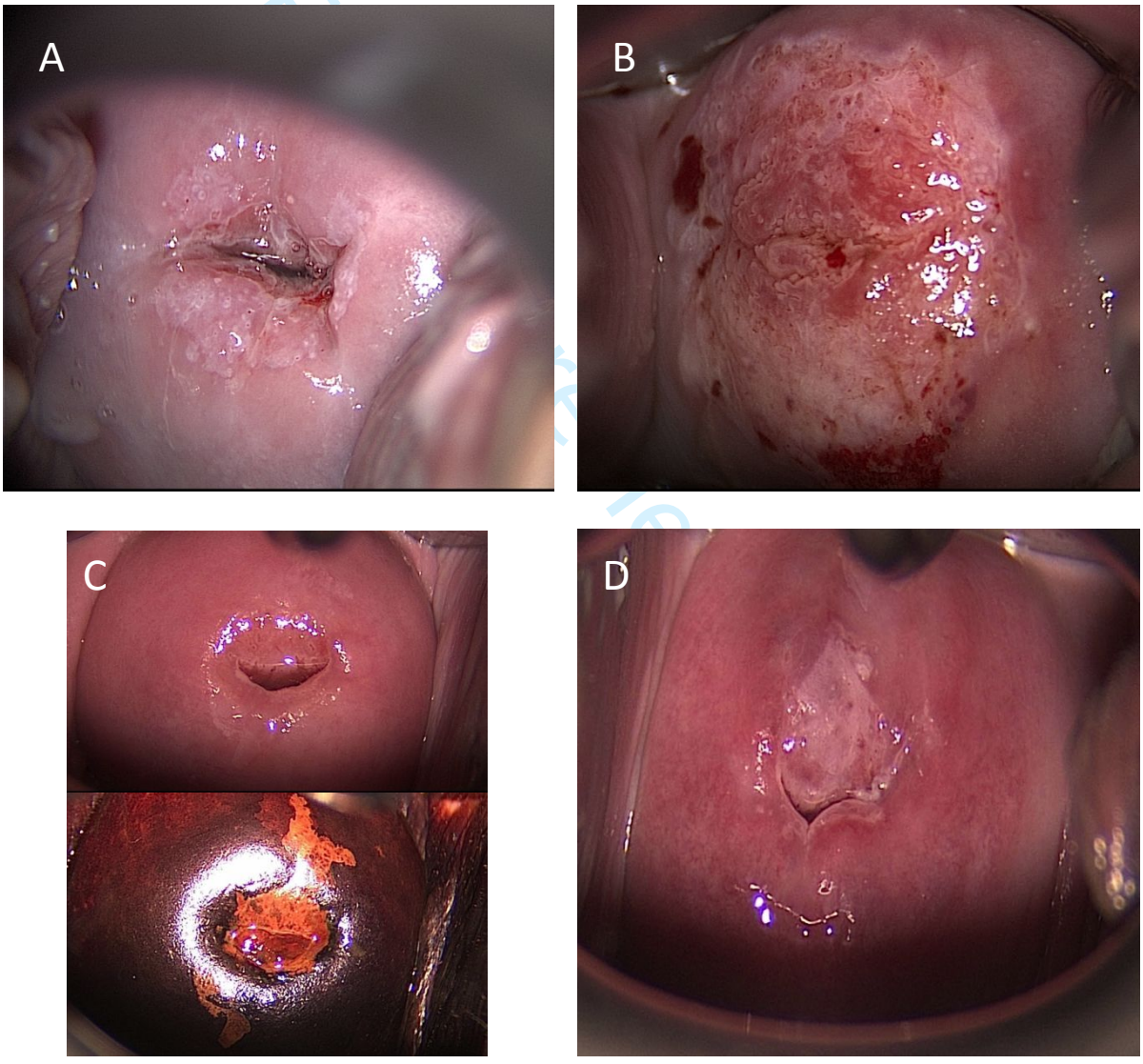
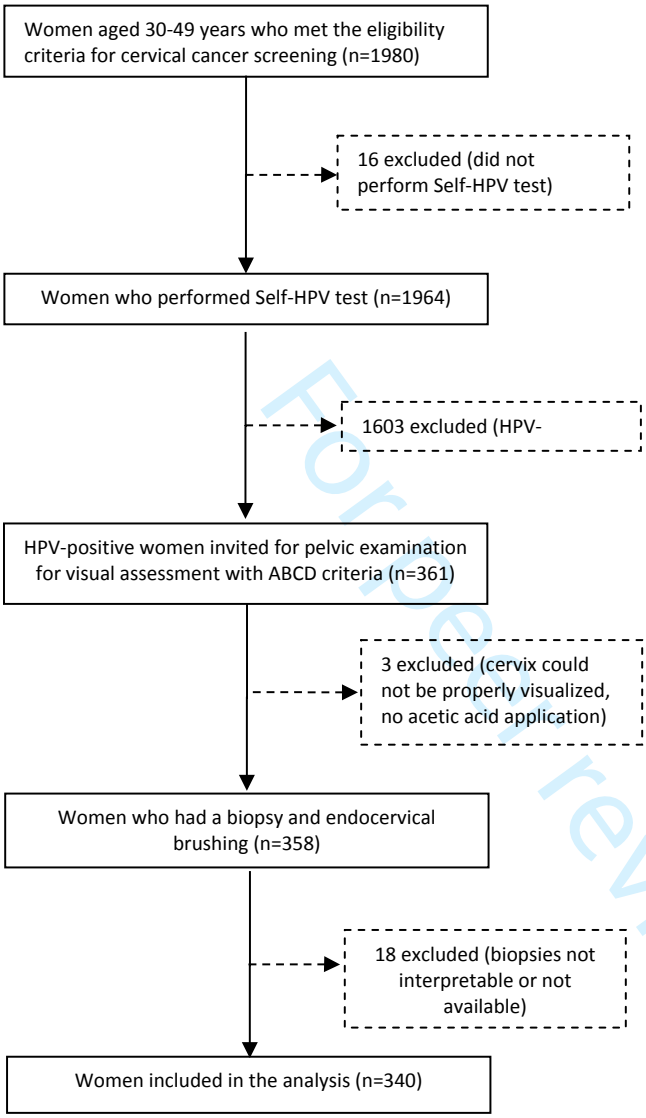


Figure 2: Flowchart of participants for the 3T-Study in Cameroon

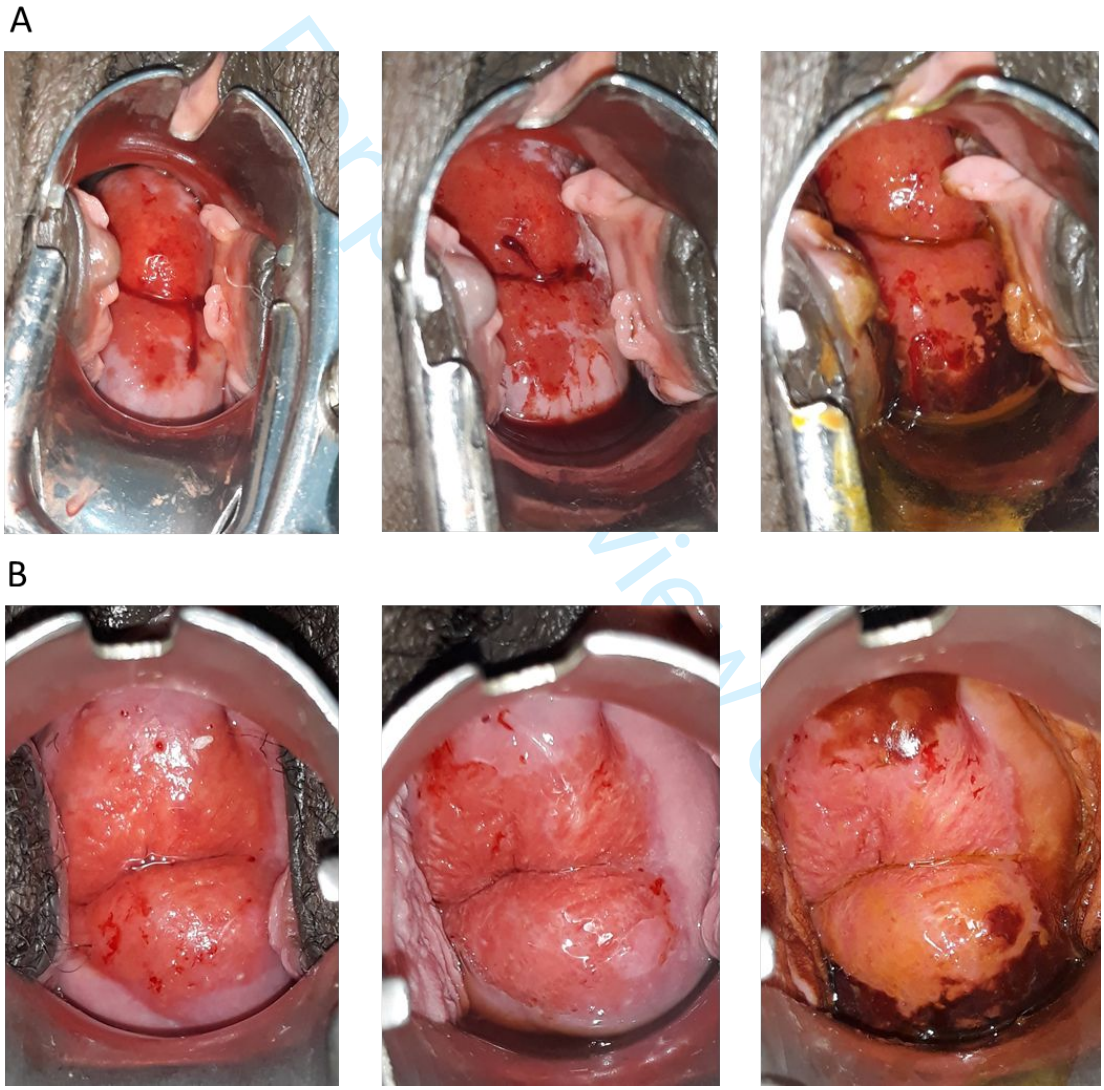


Supplementary Material

ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women: a prospective analysis

Patrick Petignat, Bruno Kenfack, Ania Wisniak, Essia Saiji, Jean-Christophe Tille, Jovanny Tsuala Fouogue, Rosa Catarino, Evelyn Foguem Tincho and Pierre Vassilakos

Figure S1. Cases of cervical cancer not identified by ABCD criteria on site



A. Poorly differentiated carcinoma, positive for criterion B (bleeding); B. Invasive adenocarcinoma, positive for criterion B. From left to right, smartphone photos of (i) the native cervix, (ii) after application of acetic acid and (iii) after application of Lugol's iodine.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**6**	Eligibility criteria	5
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	6 + figure 1
	10b	Reference standard, in sufficient detail to allow replication	7
	11	Rationale for choosing the reference standard (if alternatives exist)	na
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8
	15	How indeterminate index test or reference standard results were handled	8
	16	How missing data on the index test and reference standard were handled	8
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	na
	18	Intended sample size and how it was determined	8
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 2
	20	Baseline demographic and clinical characteristics of participants	9
	21a	Distribution of severity of disease in those with the target condition	10-11
	21b	Distribution of alternative diagnoses in those without the target condition	na
	22	Time interval and any clinical interventions between index test and reference standard	na
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	10 (table 1)
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	12 (table 3)
	25	Any adverse events from performing the index test or the reference standard	10
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	15
	27	Implications for practice, including the intended use and clinical role of the index test	14-15
OTHER INFORMATION			
	28	Registration number and name of registry	9
	29	Where the full study protocol can be accessed	9
	30	Sources of funding and other support; role of funders	16

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

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ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women: a Prospective Study of Diagnostic Accuracy

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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Global health, Diagnostics, Sexual health
Keywords:	Public health < INFECTIOUS DISEASES, EDUCATION & TRAINING (see Medical Education & Training), Community gynaecology < GYNAECOLOGY, Gynaecological oncology < GYNAECOLOGY, PREVENTIVE MEDICINE

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ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women: a Prospective Study of Diagnostic Accuracy

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ABSTRACT

Objectives A simple system for visual inspection with acetic acid (VIA) assessment, named ABCD criteria, has been developed to increase accuracy for triaging of high-risk human papillomavirus (HPV)-positive women. The present study aimed to determine the accuracy of ABCD criteria for the detection of histologically confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in HPV-positive women living in a low-resource setting.

Design Prospective study of diagnostic accuracy

Setting Cervical cancer screening program based on a 3T-Approach (Test, Triage, and Treat) in the Health District of Dschang, West Cameroon.

Participants Asymptomatic non-pregnant women aged 30-49 years were eligible to participate. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. A total of 1980 women were recruited (median age, 40 years; interquartile range, 35–45 years), of whom 361 (18·4%) were HPV-positive and 340 (94·2%) completed the trial.

Interventions HPV-positive women underwent a pelvic examination for visual assessment of the cervix according to ABCD criteria. The criteria comprised A for Acetowhiteness, B for Bleeding, C for Colouring, and D for Diameter. The ABCD criteria results were codified as positive or negative and compared with histological analysis findings (reference standards).

Primary outcome measure Diagnostic performance of ABCD criteria for CIN2+, defined as sensitivity, specificity, negative and positive predictive values.

Results ABCD criteria had a sensitivity of 77·5% (95% CI, 61·3%–88·2%), specificity of 42·0% (95% CI, 36·5%–47·7%), positive predictive value of 15·1% (95% CI, 10·8%–20·8%), and negative predictive value of 93.3% (95% CI, 87·6%–96·5%) for detection of CIN2+ lesions. Most (86·7%) of the ABCD-positive women were treated on the same day.

Conclusions ABCD criteria can be used in the context of a single-visit approach and may be the preferred triage method for management of HPV-positive women in a low-income context.

Trial registration The trial was registered under ClinicalTrials.gov (number NCT03757299).

Key words: cervical cancer screening, low- and middle-income countries, visual inspection with acetic acid (VIA), visual inspection with Lugol's iodine (VILI), human papillomavirus (HPV), triage

Strengths and limitations of this study

- Using ABCD criteria for VIA interpretation is a simple test with binary results (positive or negative) that are immediately available, allowing a screen-and-treat approach .

- Because all HPV-positive women underwent biopsy and endocervical curettage regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity.
- A limitation of the study was its setting in a single centre in a district hospital in West Cameroon with five clinicians administering all screening and treatment procedures.

INTRODUCTION

More than 90% of cervical cancer (CC) deaths occur in low- and middle-income countries (LMICs), mainly due to lack of prevention.(1) Cytology-based CC screening programs and more recent HPV-based programs have been successfully implemented in high-income countries and have been associated with important reductions in deaths from CC.(2) However, these strategies have not been implemented in LMICs, predominantly because of financial and logistical limitations. Alternative methods such as visual inspection of the cervix after application of acetic acid (VIA) and more recently, HPV primary screening, are considered suitable for use in LMICs.(3,4)

A global strategy for the elimination of cervical cancer has been launched by the World Health Organization (WHO) in 2020, which relies upon the screening of 70% of women using a high-performance test and the treatment of 90% of women identified with cervical disease.(5) Recommendations adopted by the WHO for screening in resource-limited settings include a strategy of HPV-screening followed by VIA triage and treatment, or a strategy of HPV-screening followed by treatment.(3) Although no recommendations are given for the approach that should be prioritized, sub-Saharan Africa has a high HPV prevalence rate of 15%–30% and most HPV-positive women have no lesions.(3,6,7) In this context, HPV testing followed by immediate treatment can represent significant overtreatment in women

with an HPV-positive test, which by itself may not confer a high risk of cervical intraepithelial neoplasia grade 2 or worse (CIN2+).(4,8,9) In sub-Saharan Africa, the prevalence of CIN2+ was reported to be 2%–4% in women aged 30–49 years and 7%–11% in an HPV-positive population with a low HIV prevalence rate (<10%).(6,7,10) A triage system is only a valid option if it can improve the positive predictive value (PPV) for CIN2+ and minimize the referral rate, while conserving the high sensitivity of the HPV test. The achievement of a high PPV at the cost of limited sensitivity may be considered a reasonable option when the loss to follow-up of women requiring surveillance is minimal. However, in low-resource settings, high levels of loss to follow-up constitute an important barrier to cervical cancer screening, which is why programs having no follow-up visits or as few as possible are preferable to achieve a high degree of participation.(11)

Triage by VIA and/or visual inspection with Lugol's iodine (VILI) requires accurate criteria to decide whether or not the findings are positive, which are generally based on the International Agency for Research against Cancer (IARC) manual.(12) However, in this setting, VIA triage in HPV-positive populations appears to be associated with an important loss of sensitivity, suggesting that triage by VIA using traditional criteria may not be of benefit.(6,7,10,13) Previous studies using histology as reference standard and having excluded verification bias had sensitivities ranging from 25.0% to 45.5%.(6,10,14)

Interpreting VIA with naked eye alone is subjective and is highly variable between health care providers.(15–17) This issue may be improved with continuous supervision and medical education thanks to the use of digital VIA and VILI (D-VIA/D-VILI). This includes acquisition of cervical images, native and after VIA and VILI application, through a camera or smartphone. These technologies provide an alternative to colposcopy in the context of LMICs and may constitute an important step in the improvement of VIA/VILI interpretation.(18–20) Although the image quality is probably lower than that with high-resolution colposcopy, there are significant benefits for healthcare providers, because they can move through and compare the native, VIA, and VILI images, and can also magnify suspicious lesions, before deciding whether treatment is needed.(18,19)

To improve VIA/D-VIA interpretation as a triage test in HPV-positive populations, we introduced a set of criteria, termed ABCD criteria for “Acetowhiteness”, “Bleeding”, “Colouring” (with Lugol’s iodine) and “Diameter” of the lesion. These criteria constitute a simple structure that may contribute to preventing CC in an LMIC context. The aim of the present study was to provide a rationale for the ABCD criteria and determine their performance in identifying histology-proven CIN2+.

METHODS

Study design – This prospective study was carried out between September 2018 and March 2020 in the health district of Dschang (West Cameroon) as part of a 5-year cervical cancer screening programme. The screening strategy consisted of the “3T-Approach”, in which Testing with HPV, Triage with VIA and Treatment are provided within one visit.

Asymptomatic non-pregnant women aged 30-49 years were eligible to participate in the study on a voluntary basis and were included in a consecutive manner upon presentation to the screening site. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. The study was conducted within a larger trial aiming to recruit 6,000 women in a 5-year screening program.(20) At the baseline visit, after obtaining written informed consent and providing guidance to participants on the procedure for vaginal self-sampling, participants undertook an HPV self-test (Self-HPV) that was subsequently analyzed by a point-of-care assay (GeneXpert®) in one hour. HPV-negative women were reassured and advised to repeat the test in 5 years, while HPV-positive women were invited to undergo visual triage and thermal ablation or large loop excision of the transformation zone (LLETZ) if needed. Healthcare providers performed gynecologic examination with VIA/VILI, assessment of ABCD criteria and transformation zone (TZ) type, and determined treatment modalities in a single visit.

ABCD criteria (Figure 1) – The ABCD criteria were chosen from a synthesis of published results as well as our own experience in VIA and VILI interpretation.(3,12,21–25) We considered acetowhiteness as the most important predictor for CIN and noted that Lugol's iodine can be used to identify thin acetowhite lesions not seen on the initial VIA assessment (Figure 1). Similar to the IARC criteria, the pathological area should be located within or in contact with the TZ. The ABCD criteria are codified as positive (present) or negative (absent). To be considered ABCD-positive, at least one of the following conditions needs to be fulfilled: presence of criteria A (acetowhiteness) and D (diameter) combined, or criterion B (bleeding) with or without presence of A, C (colouring) or D.

ABCD criteria were independently evaluated by one of three trained midwives and supervised by two experienced Cameroonian gynaecologists..

- *Criterion A for **A**cetowhiteness* – Criterion A is obtained after application of 3%–5% acetic acid. Any acetowhite area touching the TZ and having a diameter of >5 mm (criterion D) is considered positive. Compared with the IARC criteria, which require a degree of whiteness combined with the presence of a sharp, distinct, well defined, dense (opaque/dull or oyster white) acetowhite area,(12) we considered here any acetowhite lesion exceeding 5 mm to be positive.
- *Criterion B for **B**leeding on touch* – Criterion B is obtained upon native examination or after acetic acid application. Presence of cervical bleeding without touching or after lightly touching the cervix in the TZ area is considered positive. This means that any bleeding from the surface of the cervix, after excluding bleeding of intra-uterine origin, can be associated with CIN2+ lesions. Although bleeding can also be caused by ulceration or

infection, any signs should be thoroughly investigated to rule out the possibility of early preclinical invasive cancer. This sign is easy to recognize and is considered a high-risk finding for precancerous lesions and cervical cancer.(24,25) Presence of bleeding in association with criteria A and C may require referral for further testing like biopsy and colposcopy.

- *Criterion C for **C**olouring with Lugol's iodine* – Criterion C is optional. Lugol's iodine staining can be used as an adjunct to VIA to recognize epithelial change that would otherwise be difficult to identify by VIA only. The colour changes with VILI can be easier to appreciate than those after VIA and may contribute to identification of a missed thin acetowhite lesion. To be considered positive, an iodine-negative lesion should correspond to a VIA lesion having criteria A and D. Compared with the IARC criteria, which require the presence of a well-defined, bright yellow, iodine non-uptake area,(12) we consider any non-iodine uptake areas to be positive, providing they match an acetowhite lesion.
- *Criterion D for **D**iameter* – Criterion D is evaluated after application of acetic acid (or Lugol's iodine). An acetowhite lesion measuring >5 mm in diameter (about the size of a pencil eraser) is considered positive. Defining a minimal size of 5 mm allows exclusion of benign conditions such as dot-like, line-like, or streak-like areas.(23)

A set of three images (native, acetic acid, Lugol's iodine) were obtained on a Galaxy S5 smartphone (Samsung, Seoul, South Korea). Diagnosis and treatment were based on combined results of VIA/VILI and smartphone-enhanced D-VIA, using aids such as zooming in on lesions and performing comparisons between the native, VIA, and VILI images.

Eligiblity criteria for thermal ablation were women being positive for ABCD criteria.

Indications for referral to determine further treatment modalities were (i) lesions extending

into the endocervix which could not be covered by the probe tip, (ii) suspicion of carcinoma, in-situ adenocarcinoma or invasive adenocarcinomaOur management of HPV-positive women with a TZ type 3 was as follows: (i) those having no lesion on visual assessment were offered follow-up, (ii) those having a lesion which could be covered by thermal ablation tips were treated, and (iii) those with an endocervical lesion which could not be fully covered by the probe were referred for LLETZ. Cervical liquid-based cytology, biopsy at the TZ and endocervical curettage (ECC) were performed on all HPV-positive women prior to treatment.

Cytology – Cervical liquid-based cytology was performed using the SurePath (September 2018 to July 2019) and ThinPrep (July 2019 to March 2020) techniques. All vials were analyzed in Switzerland (CytoPath, Unilabs, Geneva, and University Hospital of Geneva). The slides were independently read by qualified cytotechnologists and classified according to the Bethesda classification system: negative for intraepithelial lesion or malignancy (NILM), inflammatory atypical squamous cells of undetermined significance (ASC-US), inflammatory atypical squamous cells that cannot exclude HSIL (ASC-H), atypical glandular cells with low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and invasive cancer.

Histology findings (reference standard) – Cervical biopsies were performed using biopsy forceps, and ECC was carried out with an endocervical brush. Cervical biopsies were

performed at 6 o'clock in the TZ when ABCD criteria were negative. If ABCD criteria were positive, one or more biopsies were performed at the most suspicious areas. All samples were stored in formalin. Biopsy slides and ECC samples (processed by cellular block) were read by two experienced gynaecologic pathologists of the Geneva University Hospitals, Switzerland, who were blinded to the screening test results and ABCD criteria findings. There was no external review of histological analyses. The histological results were classified as normal, CIN1, CIN2, CIN3, adenocarcinoma *in situ* (AIS), invasive carcinoma, or adenocarcinoma. The cut-off for a pathological result was set at CIN2+. When histological results varied within the samples of one participant, only the worst result was considered as the reference standard.

Patient and public involvement – Preferences of and experience with former patients of a preliminary research study on cervical cancer screening in Dschang, Cameroon, were considered in the design and conduction of this study. During the study, focus groups were organized with members of the community (women and men), health care workers and community health workers, to explore barriers to cervical cancer screening and further improve the program and recruitment strategy. Patients were also involved at their arrival at the screening center where they were offered a one-hour information session on cervical cancer and sexual health by trained midwives. Furthermore, the public is kept informed about

the progress of our research through the publication of yearly newsletters disseminated among health workers and the general community.

Statistical analysis – Initially, we planned a sample of 6,000 women. However, the COVID-19 pandemic and public health measures to control the virus have impacted on-site clinical activity since mid-March 2020. In this context, we decided to consider an interim analysis to the trial of the primary endpoints which included performance of the ABCD criteria. Descriptive statistics were used to analyse the baseline characteristics of the study population. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) plus their 95% confidence intervals (95% CIs) were calculated. Student's *t*-test, Mann–Whitney test, or Pearson's chi-square test were used, where appropriate, to identify sociodemographic and reproductive characteristics of the patients that could differ between ABCD criteria results. A P-value of <0·05 was considered statistically significant. An exploratory analysis was performed to assess the relationships between each independent variable and the correct prediction of the ABCD criteria. This correct prediction score was equal to 1 when ABCD criteria were positive and there was a CIN2+ on histology or if the ABCD criteria were negative and histology was also negative. All other incorrect predictions were assigned the value 0. Univariate and multivariate logistic regression analyses were carried out to identify predictors of a correct ABCD criteria score according to histology.

Participants with missing or indeterminate results for ABCD criteria or histopathology were excluded from the analysis. Odds ratios (ORs) were adjusted for potential confounders, such as age, marital status, number of lifetime sexual partners, age at first sexual intercourse, age at first delivery, parity, HIV status, and type of TZ, and 95% CIs were calculated. All data analyses were conducted using Stata Statistical software Release 13 (StataCorp LP, College Station, TX).

Ethical considerations – The study obtained approval from the Cantonal Ethics Board of Geneva, Switzerland (Commission cantonale d'éthique de la recherche [CCER], No. 2017-0110) and the Cameroonian National Ethics Committee for Human Health Research (No. 2018/07/1083/CE/CNERSH/SP). The trial was registered under ClinicalTrials.gov (number NCT03757299). The full study protocol can be provided upon request to the first author.

RESULTS

A total of 1980 women aged 30–49 years were enrolled (median age: 41 years; interquartile range [IQR], 36–50 years). Overall, 1964 women performed Self-HPV, of whom 361 (18·5%) had an HPV-positive test and underwent pelvic examination, three were excluded from the results analysis for lack of ABCD criteria assessment, and 340 (94·2%) had interpretable histology findings and constituted the study population (**Figure 2**). **Table 1** provides details of

the baseline sociodemographic, reproductive, and clinical characteristics of the participants. Median age at first sexual intercourse was 18 years (IQR, 16–19 years) and median number of sexual lifetime partners was 3 (IQR, 2–5).

Table 1: Baseline sociodemographic, reproductive health, and clinical characteristics according to ABCD criteria (N=358)*

Variable	ABCD criteria-negative	ABCD criteria-positive	Total	P-value
Participants recruited, n (%)	140 (39.1)	218 (60.9)	358	
Age (years), median (IQR)	41 (35–45)	40 (34–45)	40 (34–45)	0.4464
Marital status, n (%)				0.8910
Single	15 (10.7)	20 (9.2)	35 (9.8)	
With partner	109 (77.9)	173 (79.3)	282 (78.8)	
Divorced/widowed	16 (11.4)	25 (11.5)	41 (11.4)	
Education, n (%)				0.3900
Unschooled	1 (0.7)	5 (2.3)	6 (1.7)	
Primary education	37 (26.4)	66 (30.3)	103 (28.8)	
Secondary education	67 (47.9)	105 (48.2)	172 (48.0)	
Tertiary education	35 (25.0)	42 (19.2)	77 (21.5)	
Employment status, n (%)				0.1750
Employed	50 (35.7)	57 (26.2)	107 (29.9)	
Independent	39 (27.9)	56 (25.7)	95 (26.5)	
Housewife	23 (16.4)	41 (18.8)	64 (17.9)	
Unemployed	7 (5.0)	12 (5.5)	19 (5.3)	
Farmer	21 (15.0)	52 (23.8)	73 (20.4)	
Age at menarche (years), mean ± SD	14.7±1.8	14.7±1.9	14.7±1.8	0.8914
Age at first intercourse, median (IQR)	17 (16–19)	18 (16–20)	18 (16–19)	0.2390
Number of sexual partners, median	4 (3–6)	3 (2–5)	3 (2–5)	**0.0008**
Contraception, n (%)				0.5950
None	93 (66.9)	142 (65.5)	235 (66.0)	
Condom	18 (13.0)	25 (11.5)	43 (12.1)	
Hormonal pill	1 (0.7)	7 (3.2)	8 (2.3)	
DIU/ implant/ injection	25 (18.0)	41 (18.9)	66 (18.5)	
Other	2 (1.4)	2 (0.9)	4 (1.1)	
HIV status, n (%)				0.9420
Negative	128 (92.7)	198 (93.0)	326 (92.9)	
Positive	10 (7.3)	15 (7.0)	25 (7.1)	
Age at first delivery (years), mean ± SD	21.4±3.7	21.4±2.5	21.4±3.8	0.9137
Parity, n (%)				**0.0080**
Nulliparous	11 (7.9)	3 (1.4)	14 (3.9)	
1–4	66 (47.1)	108 (49.5)	174 (48.6)	
>4	63 (45.0)	107 (49.1)	170 (47.5)	
Transformation zone, n (%)				**<0.0001**
TZ1	76 (57.1)	150 (73.5)	226 (67.1)	
TZ2	26 (19.6)	45 (22.1)	71 (21.1)	
TZ3	31 (23.3)	9 (4.4)	40 (11.8)	
HPV testing results, n (%)				
HPV-16	11 (7.9)	23 (10.6)	34 (9.5)	0.3890

HPV-18/45	22 (15.8)	31 (14.2)	53 (14.9)	0.6770
Other HPV	114 (82.0)	186 (85.3)	300 (84.0)	0.4060
Cytology, n (%) (Total= 343)				0.0990
Normal	108 (82.5)	161 (75.9)	269 (78.4)	
ASC-US	7 (5.3)	10 (4.7)	17 (5.0)	
LSIL	10 (7.6)	15 (7.1)	25 (7.3)	
HSIL	4 (3.1)	21 (9.9)	25 (7.3)	
ASC-H	0	4 (1.9)	4 (1.2)	
Cancer	2 (1.5)	1 (0.5)	3 (0.8)	
Histology, n (%) (Total=340)				**0.0040**
Normal	108 (80.0)	129 (62.9)	237 (69.7)	
CIN1	18 (13.3)	45 (21.9)	63 (18.5)	
CIN2	1 (0.7)	12 (5.9)	13 (3.8)	
CIN3	6 (4.4)	18 (8.8)	24 (7.1)	
Invasive cancer	2 (1.5)	1 (0.5)	3 (0.9)	

Abbreviations: SD = standard deviation; IQR = interquartile range; CIN1 = cervical intraepithelial neoplasia grade 1; CIN2 = cervical intraepithelial neoplasia grade 2; CIN3 = cervical intraepithelial neoplasia grade 3; HIV = human immunodeficiency virus; HPV = human papillomavirus.

*Data from the 358 participants may be missing for some variables.

Thirty-four (9·5%) samples were positive for HPV-16, 53 (14·9%) for HPV-18/45 and 300 (84·0%) for other HPV types. Overall, 218 (60·9%) participants were classified as ABCD criteria-positive. All patients positive for ABCD were treated with thermal ablation with the exception of one patient who underwent LLETZ and one patient suspicious of cancer who was biopsied and referred for multimodal therapy. Thermal ablation was provided on the same day as HPV screening in 86.7% of cases. Reasons for delaying treatment included referral for further evaluation, technical issues, bleeding at the time of screening, or choice of the patients themselves. No serious adverse event occurred as a result of the screening procedure.

Among all 358 women with HPV-positive results, 343 samples with valid cytological results and 340 samples with valid histological results were obtained. Of the 343 valid cytological

results, 21·6% had abnormal cytology (ASC-US+). Four patients had ASC-H, 25 had HSIL, and three had cytology suggesting cancer. All three cancers identified by cytology were confirmed by histology. Of the 340 valid histological results, 63 (18·5%) CIN1 were identified, 13 (3·8%) CIN2, 24 (7·1%) CIN3, and 3 (0·9%) invasive cancers. The prevalence of CIN2+ and CIN3+ was 11·8% and 7·9%, respectively. Details for the disease prevalences are also shown in **Table 1**.

Table 2 shows demographic and pathological characteristics associated with a correct prediction of the ABCD criteria.

Table 2: Demographic and pathological characteristics associated with a correct prediction of the ABCD criteria (N=340)*

Variable	Total	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)**	P-value
Age (years), n (%)					
30–40	186 (54.7)	1.00 (Reference)		1.00 (Reference)	
41–50	154 (45.3)	1.39 (0.90–2.14)	0.133	1.51 (0.87–2.60)	0.140
Marital status, n (%)					
Single	34 (10.0)	1.00 (Reference)		1.00 (Reference)	
With partner	265 (77.9)	1.15 (0.56–2.36)	0.706	1.07 (0.43–2.63)	0.887
Divorced/widowed	41 (12.1)	0.81 (0.32–2.04)	0.656	0.63 (0.19–2.04)	0.442
Education, n (%)					
Unschooled/primary education	101 (29.7)	1.00 (Reference)		1.00 (Reference)	
Secondary/tertiary education	239 (70.3)	1.04 (0.65–1.65)	0.879	0.92 (0.47–1.82)	0.818
Employment status, n (%)					
Employed	104 (30.6)	1.00 (Reference)		1.00 (Reference)	
Independent	93 (27.3)	0.90 (0.51–1.57)	0.706	0.73 (0.38–1.43)	0.363
Housewife	58 (17.1)	0.81 (0.43–1.55)	0.528	0.74 (0.34–1.63)	0.461
Unemployed	19 (5.6)	0.72 (0.27–1.95)	0.528	0.89 (0.27–2.91)	0.852
Farmer	66 (19.4)	0.69 (0.37–1.29)	0.248	**0.41 (0.18–0.95)**	**0.037**
Age at first intercourse (years), n (%)					
≤17	154 (45.6)	1.00 (Reference)		1.00 (Reference)	
≥18	184 (54.4)	0.70 (0.46–1.08)	0.106	0.75 (0.43–1.31)	0.315
Number of sexual partners†, median	**3 (2–5)**	**1.08 (1.01–1.16)**	**0.031**	1.06 (0.97–1.17)	0.176
1–2, n (%)	98 (28.8)	1.00 (Reference)		1.00 (Reference)	
3–5, n (%)	177 (52.1)	1.39 (0.84-2.30)	0.195	1.22 (0.67-2.22)	0.506
>5, n (%)	**65 (19.1)**	**1.96 (1.04-3.70)**	**0.038**	1.53 (0.70–3.38)	0.284
Contraception, n (%)					
No	225 (66.6)	1.00 (Reference)		1.00 (Reference)	
Yes	113 (33.4)	0.84 (0.54–1.33)	0.466	0.92 (0.54–1.85)	0.769
HIV status, n (%)					
Negative	309 (92.8)	1.00 (Reference)		1.00 (Reference)	

Positive	24 (7.2)	1.21 (0.53–2.77)	0.657	0.95 (0.36–2.53)	0.589
Age at first delivery (years), n (%)					
≤20	157 (47.7)	1.00 (Reference)		1.00 (Reference)	
≥21	172 (52.3)	0.70 (0.45–1.08)	0.102	0.60 (0.34–1.07)	0.085
Parity, n (%)					
Nulliparous	14 (4.1)	1.00 (Reference)		1.00 (Reference)	
1–4	**165 (48.5)**	**0.21 (0.06–0.79)**	**0.020**	0.26 (0.02-2.91)	0.274
>4	**161 (47.4)**	**0.23 (0.06–0.86)**	**0.029**	0.28 (0.02-3.22)	0.307
Transformation zone, n (%)					
TZ1	210 (65.8)	1.00 (Reference)		1.00 (Reference)	
TZ2	70 (22.0)	1.17 (0.68–2.02)	0.575	1.24 (0.67-2.26)	0.492
TZ3	**39 (12.2)**	**6.72 (2.84–15.93)**	**<0.0001**	**6.47 (2.59-16.21)**	**<0.0001**
HPV testing results, n (%)					
Other HPV (without co-infection)	264 (77.9)	1.00 (Reference)		1.00 (Reference)	
HPV-16/18/45	75 (22.1)	1.19 (0.70–1.98)	0.514	1.18 (0.64–2.17)	0.605
Cytology, n (%)					
High-grade+***	**29 (8.9)**	**2.47 (1.11–5.49)**	**0.027**	**3.37 (1.35–8.44)**	**0.009**

Abbreviations: 95% CI = 95% confidence interval; CIN2+ = cervical intraepithelial neoplasia grade 2 or worse.

*Data from the 340 participants may be missing for some variables.

†ORs for continuous variables indicate the change in odds for an increase of one standard deviation.

**Adjusted for age, marital status, age at first intercourse, number of lifetime sexual partners, age at first delivery, parity, HIV status, and type of transformation zone.

***High-grade lesions include ASC-H, HSIL, AIS, and cancer.

Bold values are statistically significant.

ABCD criteria were more likely to be correct in the presence of TZ type 3 (aOR = 6.47; 95% CI, 2.59–16.21; P<0·001), high-grade lesions on cytology (aOR = 3.37; 95% CI, 1.35–8.44; P<0·009) and a CIN2+ on histology (aOR = 6.05; 95% CI, 2.47–14.77; P<0·001). Overall, a correct prediction of the ABCD criteria was not impacted by the multiple sociodemographic characteristics of the population in the multivariate analysis.

Performance of ABCD and cytology for detection of high-grade cervical lesions (CIN2+ and CIN3+) is shown in **Table 3**.

Table 3: Diagnostic accuracy of ABCD criteria, cytology, and HPV for detection of CIN2+ and CIN3+

	CIN2+ (N=40, 11.8%)				
Variable	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Positivity rate* % (95% CI)
ABCD criteria-positive	77.5 (61.3–88.2)	42.0 (36.5–47.7)	15.1 (10.8–20.8)	93.3 (87.6–96.5)	60.9 (55.6-65.9)
Cytology ASC-US+	80.0 (64.0–89.9)	87.5 (83.1–90.7)	47.1 (35.3–59.2)	96.9 (93.9–98.5)	21.6 (17.4-26.4
Cytology LSIL+	70.0 (53.5–82.6)	91.3 (87.4–94.1)	52.8 (39.1–66.2)	95.6 (92.4–97.5)	16.6 (12.9-21.1)
Cytology HSIL+	62.5 (46.1–76.5)	98.6 (96.3–99.5)	86.2 (67.0–95.1)	95.0 (91.8–97.0)	9.3 (6.6-13.0)
HPV-16/18/45+	37.5 (23.5–53.9)	79.9 (74.9–84.1)	20.9 (12.3–30.8)	90.5 (86.3–93.5)	23.3 (19.1-28.1)
	CIN3+ (N=27, 7.9%)				
	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	
ABCD criteria-positive	70.4 (49.6–85.2)	40.6 (35.2–46.1)	9.3 (6.0–14.1)	94.1 (88.5–97.0)	
Cytology ASC-US+	88.9 (68.9–96.7)	85.4 (80.9–89.0)	35.3 (24.7–47.6)	98.8 (96.4–99.7)	
Cytology LSIL+	81.5 (60.9–92.5)	89.7 (85.7–92.7)	41.5 (28.7–55.5)	98.2 (95.7–99.2)	
Cytology HSIL+	74.1 (53.2–87.8)	97.0 (94.3–98.4)	68.9 (49.0–83.7)	97.7 (95.2–98.9)	
HPV-16/18/45+	44.4 (26.2–64.3)	79.8 (75.0–83.9)	16.0 (9.2–26.4)	94.3 (90.8–96.6)	

* Positivity rate calculated on total HPV-positive cases (CIN threshold not applicable).

Abbreviations: CIN2+ = cervical intraepithelial neoplasia grade 2 or worse; CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; Cytology ASC-US+ = ASC-US, LSIL, ASC-H, HSIL, AIS, and cancer; Cytology LSIL+ = LSIL, ASC-H, HSIL, AIS, and cancer; Cytology HSIL+ = ASC-H, HSIL, AIS, and cancer; HPV = human papilloma virus; HPV-16/18/45+ = HPV DNA test positive for HPV-16, HPV-18, and HPV-45; 95% CI = 95% confidence interval; PPV = positive predictive value; NPV = negative predictive value.

ABCD criteria for CIN2+ detection showed a sensitivity of 77·5% (95% CI, 61·3%–88·2%), specificity of 42·0% (95% CI, 36·5%–47·7%), PPV of 15·1% (95% CI, 10·8%–20·8%), and NPV of 93·3% (95% CI, 87·6%–96·5%). Cytology-classified HSIL+ for CIN2+ detection showed lower sensitivity of 62·5% (95% CI, 46·1%–76·5%), but higher specificity of 98·6% (95% CI, 96·3%–99·5%), PPV of 86·2% (95% CI, 67·0%–95·1%), and NPV of 95·0% (95% CI, 91·8%–97·0%). Meanwhile, cytology-classified ASC-US+ showed improved sensitivity of

80·0% (95% CI, 64·0%–89·9%) and specificity of 87·5% (95% CI, 83·1%–90·7%). Screening by HPV 16/18/45 genotyping alone had a much lower sensitivity of 37·5% (95% CI, 23·5–53·9) and a specificity of 79.9% (95% CI 74·9–84·1). ABCD criteria for CIN3+ lesion identification showed a sensitivity of 70·4% (95% CI, 49·6%–85·2%), specificity of 40·6% (95% CI, 35·2%–46·1%), PPV of 9·3% (95% CI, 6·0%–14·1%), and NPV of 94·1% (95% CI, 88·5%–97·0%).

DISCUSSION

The ABCD criteria were established to improve the performance of visual-based approaches for triage of HPV-positive women. Previous studies conducted in LMICs indicated that triage using traditional VIA criteria was not satisfactory for the detection of CIN2+ lesions, as the gain in specificity when adding VIA to HPV testing was obtained at the expense of an important loss in sensitivity.(6,7,10) The challenge for VIA screeners lies in interpreting the wide variability of cervical presentations, in populations where obstetric trauma to the cervix and history of infection are frequent, and in which CIN2+ may be difficult to identify.

The most important finding of this study is that the ABCD criteria appeared to be highly sensitive for detection of high-grade lesions in an HPV-positive population. We used both (i) a magnification technique with smartphone digital imaging that allows more detailed

examination compared with naked eye alone and (ii) a lower VIA/D-VIA threshold positivity to optimize identification of lesions. The ABCD criteria provided improved VIA sensitivity for triage of HPV-positive women compared to most previous studies using a comparable methodology (histology as reference standard) (6,10,14,25,26) This can be explained by the fact that the IARC criteria require dense VIA changes before being considered positive, thus limiting their sensitivity, while a reduced positivity threshold can contribute to improved sensitivity for CIN2+ detection.(12,23)

The low specificity arises because we considered any whitening to be positive, meaning many benign conditions (metaplasia, inflammation or other benign cervical changes) could produce false-positive results for the ABCD criteria. Criterion C (VILI/D-VILI), though dependent on criteria A and D, may contribute to the high false positive rate by categorizing benign conditions as ABCD-positive through the identification of iodine-negative areas compatible with thin, transparent or patchy acetowhite lesions. The lack of association between multiple socio-demographic variables and a correct prediction of the ACBD criteria (**Table 2**) supports the generalizability of these criteria to the overall population of women aged 30 to 49 years in West Cameroon. However, the limited sample size and the fact that the study was conducted in a single center, do not allow to extend these results to the overall female population, especially considering the differences in HPV prevalence in other regions.

Compared to screening by HPV-16/18/45 genotyping without triage, the sensitivity of the ABCD criteria was much higher, at the cost of a lower specificity. PPV was also slightly lower with triage by ABCD criteria (15·1%) than with HPV genotyping (20·9%). Overall, 54·4% of normal histology results and 71·4% of CIN1 were considered ABCD criteria positive and consequently underwent unnecessary treatment. Thus, 85% (174 of 205) of women who screened positive were treated unnecessarily. However, when considering all women screened for CC, including HPV-negative, 174 were treated unnecessarily out of 1964 screened by Self-HPV, corresponding to an overall 8·9% overtreatment rate in the total population screened. Despite the low specificity, our 3T-Approach in a single visit may be acceptable in an LMIC context because it reduces cost and loss to follow-up, which are recognized barriers to effective cervical cancer screening.(11,27) Indeed, studies in Uganda(28) and South Africa(27) have shown loss to follow-up rates between 21% and 25% after the first visit, up to 50% at 24 months. Furthermore, treatment by thermal ablation is associated with very low risks of side effects and morbidity.(29) Therefore, treatment of a significant number of false-positive cases may be considered an acceptable strategy for effective control of CC in an LMIC setting and may contribute to reaching the target of the WHO's elimination initiative.(3,5) However, the use and integration of the ABCD criteria in the cervical cancer screening process warrants multidisciplinary discussion with involved

stakeholders, taking into account the local context and resources, as well as regional HPV prevalence, prevalence of CIN2+ in HPV-positive participants, level of risk including HIV prevalence, availability of treatment modalities on site, and the possibility to offer further investigation when required. According to the context, the decision to refer has consequences for the patients and the health care system, requiring additional time and resources, and increasing the risk of loss to follow-up. Recognizing the limitations of the ABCD criteria with regard to PPV and overtreatment rates, other triaging strategies merit further investigation. The use of extended HPV genotyping (HPV 16, 18, 45, 31, 33, 35, 52 and/or 58) for the triaging of HPV-positive women is one alternative that should also be explored.

The second limitation is that the study was conducted in a single centre in a district hospital in West Cameroon with five clinicians (three midwives supervised by two gynaecologists) administering all screening and treatment procedures.

It should be noted that two out of three cervical cancers were assessed as ABCD-negative on site by the frontline health care providers and did not receive immediate treatment. After reviewing the digital images of these two cases off-site, it was determined that criterion B (bleeding) was present in both cases, which should have led to a positive ABCD result (**Supplement, Figure S1**).

ABCD criteria comprise a simple tool that can alert healthcare professionals to the clinical features of CIN2+, and the use of "relaxed IARC criteria" may greatly decrease the risk of missing CIN2+ lesions. Using ABCD criteria is a simple test with binary results (positive or negative) that are immediately available, allowing initiation of therapy without delay. In our series, 86·7% of participants underwent the 3T-Approach in one day. Strengths of our study included the application of ABCD criteria upon VIA examination in real-life conditions with immediate treatment when necessary, therefore supporting the feasibility of a "screen-and-treat" strategy. Furthermore, because all HPV-positive women underwent biopsy and cervical brushing regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity for ABCD criteria.

In conclusion, ABCD criteria can improve CIN2+ diagnosis in HPV-positive women and may provide a unique opportunity to improve cervical cancer screening programs in LMICs using a one-visit approach. This strategy may be particularly beneficial because the criteria are easily remembered and to use for healthcare providers.

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Competing Interests

All authors declare that they have no competing interests.

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Data access, analysis and responsibility

The principal investigator had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Data used in the study is available upon request to the first author.

Contributors

PP, BK, and PV designed the study protocol, implemented the study, oversaw the data collection, analysed the data, and drafted and revised the paper. AW and RC conducted data analysis, interpreted the data, and revised the draft paper. BK, ET, and JF trained the study staff, assumed the quality control (supervision and mentorship), supported the data collection, interpreted the data, and revised the draft paper. JCT and ES analysed the pathological specimens, interpreted the data, and revised the draft paper.

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Figure 1: ABCD criteria for VIA interpretation in HPV-positive women

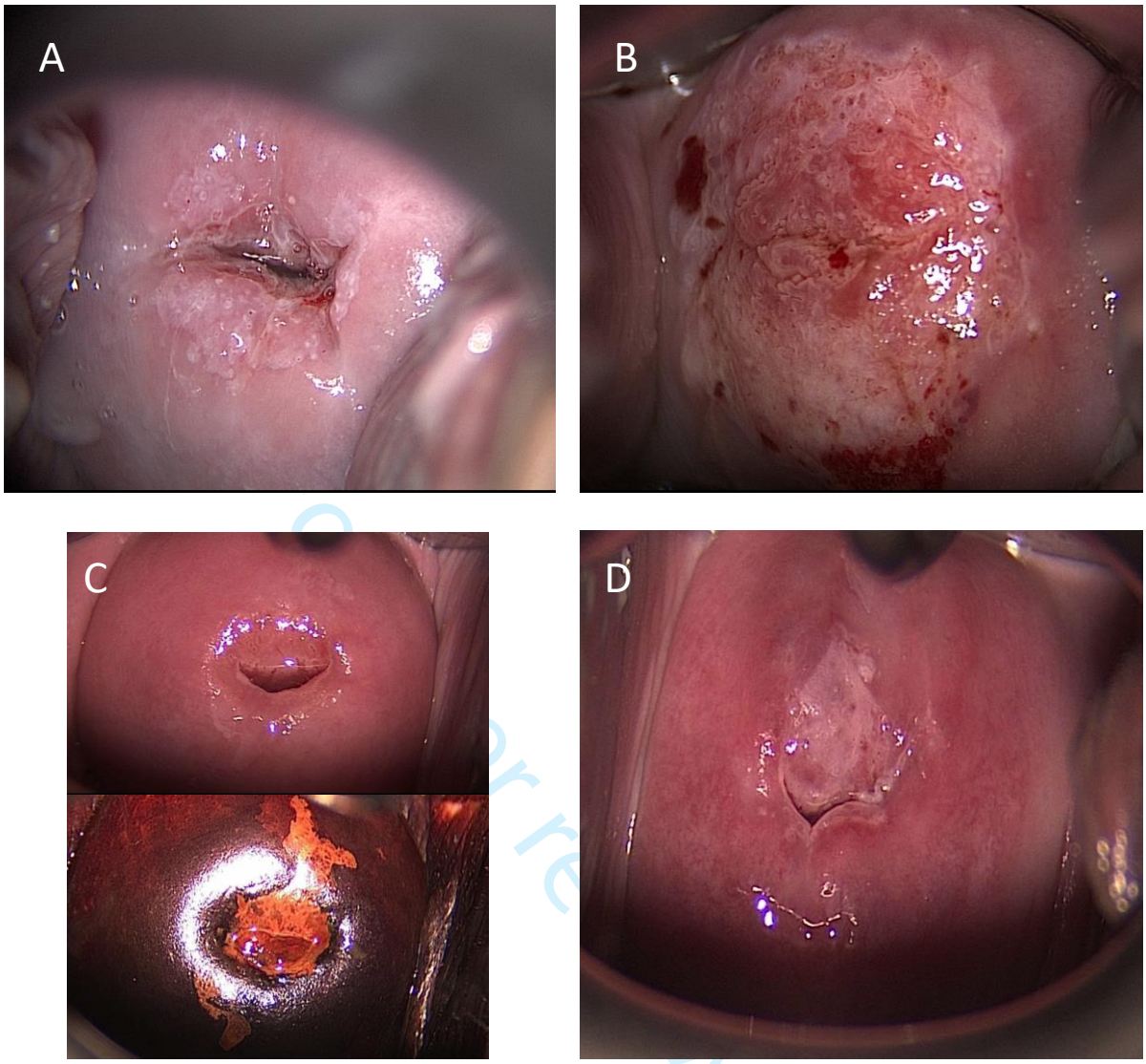
Criterion A – **A**cetowhite area touching the transformation zone (absent on the native view and apparent after acetic acid application) is considered positive.

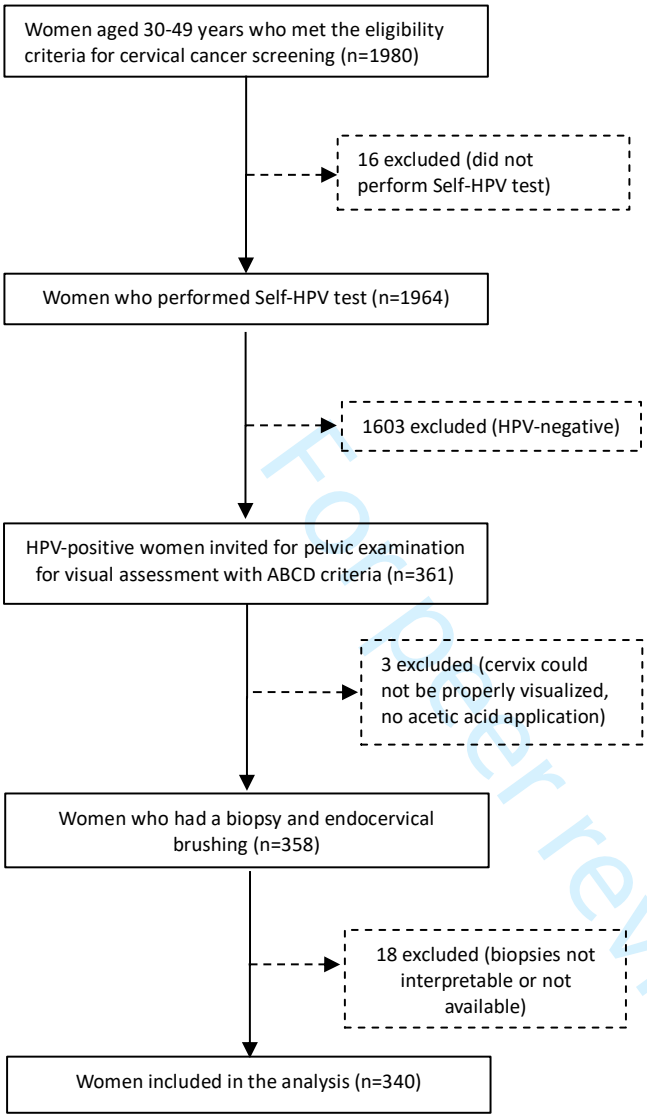
Criterion B – **B**leeding without touching or after lightly touching (with a swab or speculum) the cervix is considered positive.

Criterion C (optional) – **C**olouring with VILI contributes to confirmation or identification of a faint acetowhite lesion.

Criterion D – Diameter of >5 mm (about the size of a pencil eraser) in an acetowhite area is considered positive.

Figure 2: Flowchart of participants for the 3T-Approach in Cameroon



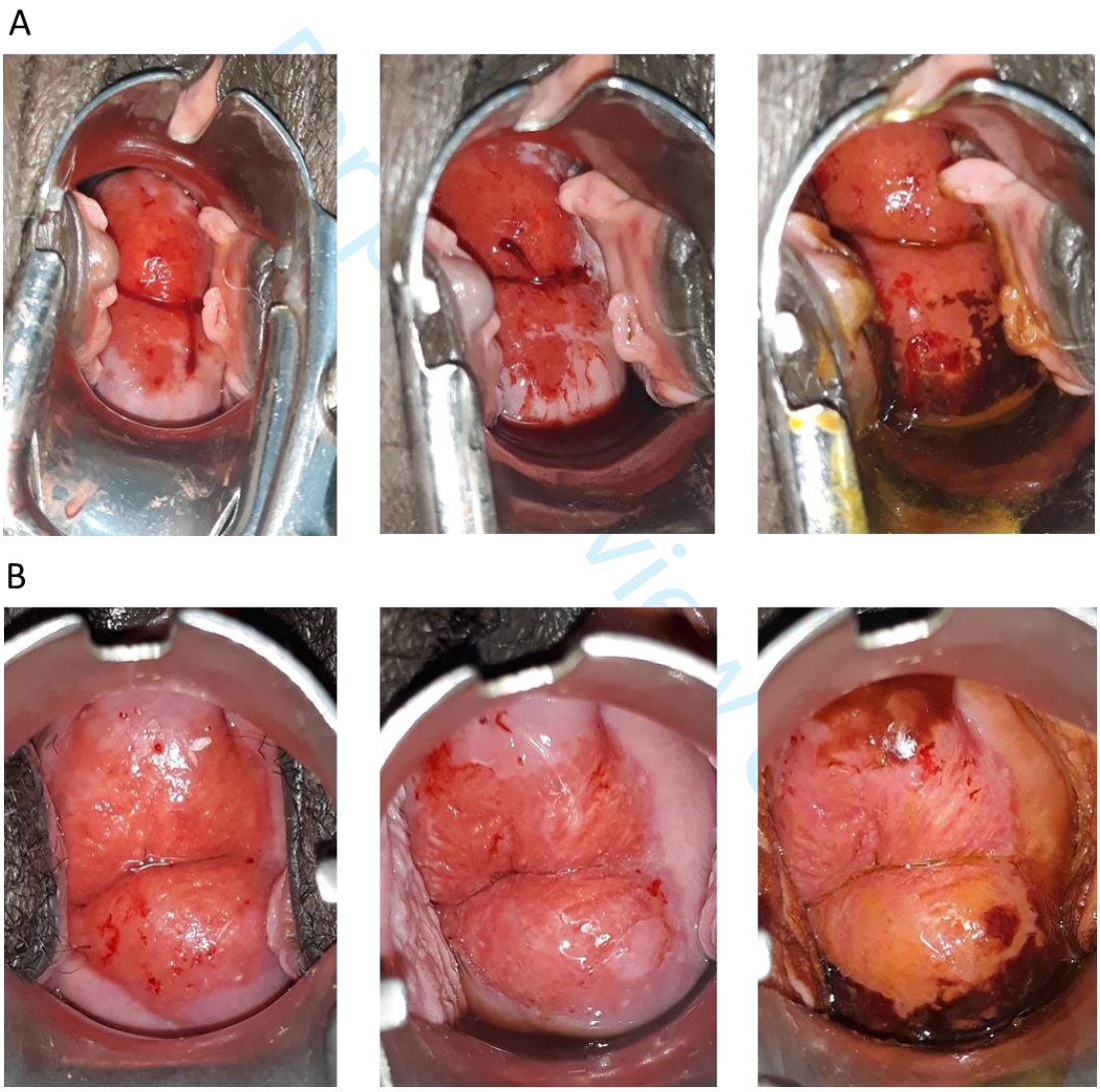


Supplementary Material

ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women: a prospective analysis

Patrick Petignat, Bruno Kenfack, Ania Wisniak, Essia Saiji, Jean-Christophe Tille, Jovanny Tsuala Fouogue, Rosa Catarino, Evelyn Foguem Tincho and Pierre Vassilakos

Figure S1. Cases of cervical cancer not identified by ABCD criteria on site



A. Poorly differentiated carcinoma, positive for criterion B (bleeding); B. Invasive adenocarcinoma, positive for criterion B. From left to right, smartphone photos of (i) the native cervix, (ii) after application of acetic acid and (iii) after application of Lugol's iodine.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**6**	Eligibility criteria	5
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	6 + figure 1
	10b	Reference standard, in sufficient detail to allow replication	7
	11	Rationale for choosing the reference standard (if alternatives exist)	na
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8
	15	How indeterminate index test or reference standard results were handled	8
	16	How missing data on the index test and reference standard were handled	8
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	na
	18	Intended sample size and how it was determined	8
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 2
	20	Baseline demographic and clinical characteristics of participants	9
	21a	Distribution of severity of disease in those with the target condition	10-11
	21b	Distribution of alternative diagnoses in those without the target condition	na
	22	Time interval and any clinical interventions between index test and reference standard	na
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	10 (table 1)
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	12 (table 3)
	25	Any adverse events from performing the index test or the reference standard	10
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	15
	27	Implications for practice, including the intended use and clinical role of the index test	14-15
OTHER INFORMATION			
	28	Registration number and name of registry	9
	29	Where the full study protocol can be accessed	9
	30	Sources of funding and other support; role of funders	16

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women: a Prospective Study of Diagnostic Accuracy

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Secondary Subject Heading:	Global health, Diagnostics, Sexual health
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ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women: a Prospective Study of Diagnostic Accuracy

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ABSTRACT

Objectives A simple system for visual inspection with acetic acid (VIA) assessment, named ABCD criteria, has been developed to increase accuracy for triaging of high-risk human papillomavirus (HPV)-positive women. The present study aimed to determine the accuracy of ABCD criteria for the detection of histologically confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in HPV-positive women living in a low-resource setting.

Design Prospective study of diagnostic accuracy

Setting Cervical cancer screening program based on a 3T-Approach (Test, Triage, and Treat) in the Health District of Dschang, West Cameroon.

Participants Asymptomatic non-pregnant women aged 30-49 years were eligible to participate. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. A total of 1980 women were recruited (median age, 40 years; interquartile range, 35–45 years), of whom 361 (18·4%) were HPV-positive and 340 (94·2%) completed the trial.

Interventions HPV-positive women underwent a pelvic examination for visual assessment of the cervix according to ABCD criteria. The criteria comprised A for Acetowhiteness, B for Bleeding, C for Colouring, and D for Diameter. The ABCD criteria results were codified as positive or negative and compared with histological analysis findings (reference standards).

Primary outcome measure Diagnostic performance of ABCD criteria for CIN2+, defined as sensitivity, specificity, negative and positive predictive values.

Results ABCD criteria had a sensitivity of 77·5% (95% CI, 61·3%–88·2%), specificity of 42·0% (95% CI, 36·5%–47·7%), positive predictive value of 15·1% (95% CI, 10·8%–20·8%), and negative predictive value of 93.3% (95% CI, 87·6%–96·5%) for detection of CIN2+ lesions. Most (86·7%) of the ABCD-positive women were treated on the same day.

Conclusions ABCD criteria can be used in the context of a single-visit approach and may be the preferred triage method for management of HPV-positive women in a low-income context.

Trial registration The trial was registered under ClinicalTrials.gov (number NCT03757299).

Key words: cervical cancer screening, low- and middle-income countries, visual inspection with acetic acid (VIA), visual inspection with Lugol's iodine (VILI), human papillomavirus (HPV), triage

Strengths and limitations of this study

- Using ABCD criteria for VIA interpretation is a simple test with binary results (positive or negative) that are immediately available, allowing a screen-and-treat approach .

- Because all HPV-positive women underwent biopsy and endocervical brushing regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity.
- A limitation of the study was its setting in a single centre in a district hospital in West Cameroon with five clinicians administering all screening and treatment procedures.

INTRODUCTION

More than 90% of cervical cancer (CC) deaths occur in low- and middle-income countries (LMICs), mainly due to lack of prevention.(1) Cytology-based CC screening programs and more recent HPV-based programs have been successfully implemented in high-income countries and have been associated with important reductions in deaths from CC.(2) However, these strategies have not been implemented in LMICs, predominantly because of financial and logistical limitations. Alternative methods such as visual inspection of the cervix after application of acetic acid (VIA) and more recently, HPV primary screening, are considered suitable for use in LMICs.(3,4)

A global strategy for the elimination of cervical cancer has been launched by the World Health Organization (WHO) in 2020, which relies upon the screening of 70% of women using a high-performance test and the treatment of 90% of women identified with cervical disease.(5) Recommendations adopted by the WHO for screening in resource-limited settings include a strategy of HPV-screening followed by VIA triage and treatment, or a strategy of HPV-screening followed by treatment.(3) Although no recommendations are given for the approach that should be prioritized, sub-Saharan Africa has a high HPV prevalence rate of 15%–30% and most HPV-positive women have no lesions.(3,6,7) In this context, HPV testing followed by immediate treatment can represent significant overtreatment in women

with an HPV-positive test, which by itself may not confer a high risk of cervical intraepithelial neoplasia grade 2 or worse (CIN2+).(4,8,9) In sub-Saharan Africa, the prevalence of CIN2+ was reported to be 2%–4% in women aged 30–49 years and 7%–11% in an HPV-positive population with a low HIV prevalence rate (<10%).(6,7,10) A triage system is only a valid option if it can improve the positive predictive value (PPV) for CIN2+ and minimize the referral rate, while conserving the high sensitivity of the HPV test. The achievement of a high PPV at the cost of limited sensitivity may be considered a reasonable option when the loss to follow-up of women requiring surveillance is minimal. However, in low-resource settings, high levels of loss to follow-up constitute an important barrier to cervical cancer screening, which is why programs having no follow-up visits or as few as possible are preferable to achieve a high degree of participation.(11) A '3T-Approach' (Test, Triage and Treat) combining testing with a rapid HPV test, triage of HPV-positive women with VIA, and treatment by thermal ablation of VIA-positive patients within the same day, has been previously used to further reduce the risk of loss to follow-up.(12)

Triage by VIA and/or visual inspection with Lugol's iodine (VILI) requires accurate criteria to decide whether or not the findings are positive, which are generally based on the International Agency for Research against Cancer (IARC) manual.(13) However, in this setting, VIA triage in HPV-positive populations appears to be associated with an important

loss of sensitivity, suggesting that triage by VIA using traditional criteria may not be of benefit.(6,7,10,14) Previous studies using histology as reference standard and having excluded verification bias had sensitivities ranging from 25.0% to 45.5%.(6,10,15)

Interpreting VIA with naked eye alone is subjective and is highly variable between health care providers.(16–18) This issue may be improved with continuous supervision and medical education thanks to the use of digital VIA and VILI (D-VIA/D-VILI). This includes acquisition of cervical images, native and after VIA and VILI application, through a camera or smartphone. These technologies provide an alternative to colposcopy in the context of LMICs and may constitute an important step in the improvement of VIA/VILI interpretation.(19–21) Although the image quality is probably lower than that with high-resolution colposcopy, there are significant benefits for healthcare providers, because they can move through and compare the native, VIA, and VILI images, and can also magnify suspicious lesions, before deciding whether treatment is needed.(19,20)

To improve VIA/D-VIA interpretation as a triage test in HPV-positive populations, we introduced a set of criteria, termed ABCD criteria for “Acetowhiteness”, “Bleeding”, “Colouring” (with Lugol’s iodine) and “Diameter” of the lesion. These criteria constitute a simple structure that may contribute to preventing CC in an LMIC context. The aim of the

present study was to provide a rationale for the ABCD criteria and determine their performance in identifying histology-proven CIN2+.

METHODS

Study design – This prospective study was carried out between September 2018 and March 2020 in the health district of Dschang (West Cameroon) as part of a 5-year cervical cancer screening programme. The screening strategy consisted of the "3T-Approach", in which Testing with HPV, Triage with VIA and Treatment are provided within one visit.

Asymptomatic non-pregnant women aged 30-49 years were eligible to participate in the study on a voluntary basis and were included in a consecutive manner upon presentation to the screening site. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. The study was conducted within a larger trial aiming to recruit 6,000 women in a 5-year screening program.(21) At the baseline visit, after obtaining written informed consent and providing guidance to participants on the procedure for vaginal self-sampling, participants undertook an HPV self-test (Self-HPV) that was subsequently analyzed by a point-of-care assay (GeneXpert®), with most results available within an hour. HPV-negative women were reassured and advised to repeat the test in 5 years, while HPV-positive women were invited to undergo visual triage and thermal ablation or large loop excision of the

transformation zone (LLETZ) if needed. Trained midwives performed gynecologic examination with VIA/VILI, assessment of ABCD criteria and transformation zone (TZ) type, and determined treatment modalities in a single visit. Two gynaecologists were available on call for a second opinion or advice.

ABCD criteria (Figure 1) – The ABCD criteria were chosen from a synthesis of published results as well as our own experience in VIA and VILI interpretation.(3,13,22–26) We considered acetowhiteness as the most important predictor for CIN and noted that Lugol's iodine can be used to identify thin acetowhite lesions not seen on the initial VIA assessment (Figure 1). Similar to the IARC criteria, the pathological area should be located within or in contact with the TZ. The ABCD criteria are codified as positive (present) or negative (absent). To be considered ABCD-positive, at least one of the following conditions needs to be fulfilled: presence of criteria A (acetowhiteness) and D (diameter) combined, or criterion B (bleeding) with or without presence of A, C (colouring) or D.

ABCD criteria were independently evaluated by one of three trained midwives and supervised by two experienced Cameroonian gynaecologists.

- *Criterion A for **A**cetowhiteness* – Criterion A is obtained after application of 3%–5% acetic acid. Any acetowhite area touching the TZ and having a diameter of >5 mm (criterion D) is considered positive. Compared with the IARC criteria, which require a degree of whiteness combined with the presence of a sharp, distinct, well defined, dense

(opaque/dull or oyster white) acetowhite area,(13) we considered here any acetowhite lesion exceeding 5 mm to be positive.

- *Criterion B for **B**leeding on touch* – Criterion B is obtained upon native examination or after acetic acid application. Presence of cervical bleeding without touching or after lightly touching the cervix in the TZ area is considered positive. This means that any bleeding from the surface of the cervix, after excluding bleeding of intra-uterine origin, can be associated with CIN2+ lesions. Although bleeding can also be caused by ulceration or infection, any signs should be thoroughly investigated to rule out the possibility of early preclinical invasive cancer. This sign is easy to recognize and is considered a risk finding for precancerous lesions and cervical cancer.(25,26) Presence of bleeding in association with criteria A and C may require referral for further testing like biopsy and colposcopy.
- *Criterion C for **C**olouring with Lugol's iodine* – Criterion C is optional. Lugol's iodine staining can be used as an adjunct to VIA to recognize epithelial change that would otherwise be difficult to identify by VIA only. The colour changes with VILI can be easier to appreciate than those after VIA and may contribute to identification of a missed thin acetowhite lesion. To be considered positive, an iodine-negative lesion should correspond to a VIA lesion having criteria A and D. Compared with the IARC criteria, which require the presence of a well-defined, bright yellow, iodine non-uptake area,(13) we consider any non-iodine uptake areas to be positive, providing they match an acetowhite lesion.
- *Criterion D for **D**iameter* – Criterion D is evaluated after application of acetic acid (or Lugol's iodine). An acetowhite lesion measuring >5 mm in diameter (about the size of a pencil eraser) is considered positive. Defining a minimal size of 5 mm allows exclusion of benign conditions such as dot-like, line-like, or streak-like areas.(24)

A set of three images (native, acetic acid, Lugol's iodine) were obtained on a Galaxy S5 smartphone (Samsung, Seoul, South Korea). Diagnosis and treatment were based on

combined results of VIA/VILI and smartphone-enhanced D-VIA, using aids such as zooming in on lesions and performing comparisons between the native, VIA, and VILI images. Women with positive ABCD criteria were eligible for treatment by thermal ablation, with the exception of (i) lesions extending into the endocervix which could not be covered by the probe tip, and (ii) suspicions of carcinoma, in-situ adenocarcinoma or invasive adenocarcinoma, which were referred to a gynaecologist to determine the need for further treatment (LLETZ or oncological management). Cervical liquid-based cytology, biopsy at the TZ and endocervical brushing (ECB) were performed on all HPV-positive women prior to treatment.

Cytology – Cervical liquid-based cytology was performed using the SurePath (September 2018 to July 2019) and ThinPrep (July 2019 to March 2020) techniques. All vials were analyzed in Switzerland (CytoPath, Unilabs, Geneva, and University Hospital of Geneva). The slides were independently read by qualified cytotechnologists and classified according to the 2014 Bethesda classification system: negative for intraepithelial lesion or malignancy (NILM), inflammatory atypical squamous cells of undetermined significance (ASC-US), inflammatory atypical squamous cells that cannot exclude HSIL (ASC-H), atypical glandular cells with low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and invasive cancer. The cytotechnologists were aware of the

HPV-positive status (but not of the HPV type) of participants but were blinded to the ABCD criteria interpretation.

Histology findings (reference standard) – Cervical biopsies were performed using biopsy forceps, and ECB was carried out with an endocervical brush. Cervical biopsies were performed at 6 o'clock in the TZ when ABCD criteria were negative. If ABCD criteria were positive, one or more biopsies were performed at the most suspicious areas. All samples were stored in formalin. Biopsy slides and ECB samples (processed by cellular block) were read by two experienced gynaecologic pathologists of the Geneva University Hospitals, Switzerland, who were blinded to the screening test results and ABCD criteria findings. There was no external review of histological analyses. The histological results were classified as normal, CIN1, CIN2, CIN3, adenocarcinoma *in situ* (AIS), invasive carcinoma, or adenocarcinoma. The cut-off for a pathological result was set at CIN2+. When histological results varied within the samples of one participant, only the worst result was considered as the reference standard.

Patient and public involvement – Preferences of and experience with former patients of a preliminary research study on cervical cancer screening in Dschang, Cameroon, were considered in the design and conduction of this study. During the study, focus groups were organized with members of the community (women and men), health care workers and

community health workers, to explore barriers to cervical cancer screening and further improve the program and recruitment strategy. Patients were also involved at their arrival at the screening center where they were offered a one-hour information session on cervical cancer and sexual health by trained midwives. Furthermore, the public is kept informed about the progress of our research through the publication of bi-annual newsletters disseminated among health workers and the general community. Newsletters will be published until the end of the 3T study.

Statistical analysis – Initially, we planned a sample of 6,000 women. However, the COVID-19 pandemic and public health measures to control the virus have impacted on-site clinical activity since mid-March 2020. In this context, we decided to consider an interim analysis to the trial of the primary endpoints which included performance of the ABCD criteria. Descriptive statistics were used to analyse the baseline characteristics of the study population. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positivity rate plus their 95% confidence intervals (95% CIs) were calculated for each triaging test. Student's *t*-test, Mann–Whitney test, or Pearson's chi-square test were used, where appropriate, to identify sociodemographic and reproductive characteristics of the patients that could differ between ABCD criteria results. A P-value of <0·05 was considered statistically significant. An exploratory analysis was performed to assess the relationships

between each independent variable and the correct prediction of the ABCD criteria. This correct prediction score was equal to 1 when ABCD criteria were positive and there was a CIN2+ on histology or if the ABCD criteria were negative and histology was also negative. All other incorrect predictions were assigned the value 0. Univariate and multivariate logistic regression analyses were carried out to identify predictors of a correct ABCD criteria score according to histology. Participants with missing or indeterminate results for ABCD criteria or histopathology were excluded from the analysis. Odds ratios (ORs) were adjusted for potential confounders, such as age, marital status, number of lifetime sexual partners, age at first sexual intercourse, age at first delivery, parity, HIV status, and type of TZ, and 95% CIs were calculated. All data analyses were conducted using Stata Statistical software Release 13 (StataCorp LP, College Station, TX).

Ethical considerations – The study obtained approval from the Cantonal Ethics Board of Geneva, Switzerland (Commission cantonale d'éthique de la recherche [CCER], No. 2017-0110) and the Cameroonian National Ethics Committee for Human Health Research (No. 2018/07/1083/CE/CNERSH/SP). The trial was registered under ClinicalTrials.gov (number NCT03757299). The full study protocol can be provided upon request to the first author.

RESULTS

A total of 1980 women aged 30–49 years were enrolled (median age: 41 years; interquartile range [IQR], 36–50 years). Overall, 1964 women performed Self-HPV, of whom 361 (18·5%) had an HPV-positive test and underwent pelvic examination, three were excluded from the results analysis for lack of ABCD criteria assessment, and 340 (94·2%) had interpretable histology findings and constituted the study population (**Figure 2**). **Table 1** provides details of the baseline sociodemographic, reproductive, and clinical characteristics of the participants. Median age at first sexual intercourse was 18 years (IQR, 16–19 years) and median number of sexual lifetime partners was 3 (IQR, 2–5).

Table 1: Baseline sociodemographic, reproductive health, and clinical characteristics according to ABCD criteria (N=358)*

Variable	ABCD criteria-negative	ABCD criteria-positive	Total	P-value
Participants recruited, n (%)	140 (39.1)	218 (60.9)	358	
Age (years), median (IQR)	41 (35–45)	40 (34–45)	40 (34–45)	0.4464
Marital status, n (%)				0.8910
Single	15 (10.7)	20 (9.2)	35 (9.8)	
With partner	109 (77.9)	173 (79.3)	282 (78.8)	
Divorced/widowed	16 (11.4)	25 (11.5)	41 (11.4)	
Education, n (%)				0.3900
Unschooled	1 (0.7)	5 (2.3)	6 (1.7)	
Primary education	37 (26.4)	66 (30.3)	103 (28.8)	
Secondary education	67 (47.9)	105 (48.2)	172 (48.0)	
Tertiary education	35 (25.0)	42 (19.2)	77 (21.5)	
Employment status, n (%)				0.1750
Employed	50 (35.7)	57 (26.2)	107 (29.9)	
Independent	39 (27.9)	56 (25.7)	95 (26.5)	
Housewife	23 (16.4)	41 (18.8)	64 (17.9)	
Unemployed	7 (5.0)	12 (5.5)	19 (5.3)	
Farmer	21 (15.0)	52 (23.8)	73 (20.4)	
Age at menarche (years), mean ± SD	14.7±1.8	14.7±1.9	14.7±1.8	0.8914
Age at first intercourse, median (IQR)	17 (16–19)	18 (16–20)	18 (16–19)	0.2390
Number of sexual partners, median	4 (3–6)	3 (2–5)	3 (2–5)	**0.0008**
Contraception, n (%)				0.5950
None	93 (66.9)	142 (65.5)	235 (66.0)	
Condom	18 (13.0)	25 (11.5)	43 (12.1)	

Hormonal pill	1 (0.7)	7 (3.2)	8 (2.3)	
DIU/ implant/ injection	25 (18.0)	41 (18.9)	66 (18.5)	
Other	2 (1.4)	2 (0.9)	4 (1.1)	
HIV status, n (%)				0.9420
Negative	128 (92.7)	198 (93.0)	326 (92.9)	
Positive	10 (7.3)	15 (7.0)	25 (7.1)	
Age at first delivery (years), mean ± SD	21.4±3.7	21.4±2.5	21.4±3.8	0.9137
Parity, n (%)				**0.0080**
Nulliparous	11 (7.9)	3 (1.4)	14 (3.9)	
1–4	66 (47.1)	108 (49.5)	174 (48.6)	
>4	63 (45.0)	107 (49.1)	170 (47.5)	
Transformation zone, n (%)				**<0.0001**
TZ1	76 (57.1)	150 (73.5)	226 (67.1)	
TZ2	26 (19.6)	45 (22.1)	71 (21.1)	
TZ3	31 (23.3)	9 (4.4)	40 (11.8)	
HPV testing results, n (%)				
HPV-16	11 (7.9)	23 (10.6)	34 (9.5)	0.3890
HPV-18/45	22 (15.8)	31 (14.2)	53 (14.9)	0.6770
Other HPV	114 (82.0)	186 (85.3)	300 (84.0)	0.4060
Cytology, n (%) (Total= 343)				0.0990
Normal	108 (82.5)	161 (75.9)	269 (78.4)	
ASC-US	7 (5.3)	10 (4.7)	17 (5.0)	
LSIL	10 (7.6)	15 (7.1)	25 (7.3)	
HSIL	4 (3.1)	21 (9.9)	25 (7.3)	
ASC-H	0	4 (1.9)	4 (1.2)	
Cancer	2 (1.5)	1 (0.5)	3 (0.8)	
Histology, n (%) (Total=340)				**0.0040**
Normal	108 (80.0)	129 (62.9)	237 (69.7)	
CIN1	18 (13.3)	45 (21.9)	63 (18.5)	
CIN2	1 (0.7)	12 (5.9)	13 (3.8)	
CIN3	6 (4.4)	18 (8.8)	24 (7.1)	
Invasive cancer	2 (1.5)	1 (0.5)	3 (0.9)	

Abbreviations: SD = standard deviation; IQR = interquartile range; CIN1 = cervical intraepithelial neoplasia grade 1; CIN2 = cervical intraepithelial neoplasia grade 2; CIN3 = cervical intraepithelial neoplasia grade 3; HIV = human immunodeficiency virus; HPV = human papillomavirus.

*Data from the 358 participants may be missing for some variables.

Thirty-four (9·5%) samples were positive for HPV-16, 53 (14·9%) for HPV-18/45 and 300 (84·0%) for other HPV types. Overall, 218 (60·9%) participants were classified as ABCD criteria-positive. All patients positive for ABCD were treated with thermal ablation with the exception of one patient who underwent LLETZ and one patient suspicious of cancer who was biopsied and referred for multimodal therapy. Thermal ablation was provided on the same day as HPV screening in 86.7% of cases. Reasons for delaying treatment included

referral for further evaluation, technical issues, bleeding at the time of screening, or choice of the patients themselves. No serious adverse event occurred as a result of the screening procedure.

Among all 358 women with HPV-positive results, 343 samples with valid cytological results and 340 samples with valid histological results were obtained. Of the 343 valid cytological results, 21·6% had abnormal cytology (ASC-US+). Four patients had ASC-H, 25 had HSIL, and three had cytology suggesting cancer. All three cancers identified by cytology were confirmed by histology. Of the 340 valid histological results, 63 (18·5%) CIN1 were identified, 13 (3·8%) CIN2, 24 (7·1%) CIN3, and 3 (0·9%) invasive cancers. The prevalence of CIN2+ and CIN3+ was 11·8% and 7·9%, respectively. Details for the disease prevalences are also shown in **Table 1**.

Table 2 shows demographic and pathological characteristics associated with a correct prediction of the ABCD criteria.

Table 2: Demographic and pathological characteristics associated with a correct prediction of the ABCD criteria (N=340)*

Variable	Total	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)**	P-value
Age (years), n (%)					
30–40	186 (54.7)	1.00 (Reference)		1.00 (Reference)	
41–50	154 (45.3)	1.39 (0.90–2.14)	0.133	1.51 (0.87–2.60)	0.140
Marital status, n (%)					
Single	34 (10.0)	1.00 (Reference)		1.00 (Reference)	
With partner	265 (77.9)	1.15 (0.56–2.36)	0.706	1.07 (0.43–2.63)	0.887
Divorced/widowed	41 (12.1)	0.81 (0.32–2.04)	0.656	0.63 (0.19–2.04)	0.442
Education, n (%)					
Unschooled/primary education	101 (29.7)	1.00 (Reference)		1.00 (Reference)	
Secondary/tertiary education	239 (70.3)	1.04 (0.65–1.65)	0.879	0.92 (0.47–1.82)	0.818
Employment status, n (%)					

Employed	104 (30.6)	1.00 (Reference)		1.00 (Reference)	
Independent	93 (27.3)	0.90 (0.51–1.57)	0.706	0.73 (0.38–1.43)	0.363
Housewife	58 (17.1)	0.81 (0.43–1.55)	0.528	0.74 (0.34–1.63)	0.461
Unemployed	19 (5.6)	0.72 (0.27–1.95)	0.528	0.89 (0.27–2.91)	0.852
Farmer	66 (19.4)	0.69 (0.37–1.29)	0.248	**0.41 (0.18–0.95)**	**0.037**
Age at first intercourse (years), n (%)					
≤17	154 (45.6)	1.00 (Reference)		1.00 (Reference)	
≥18	184 (54.4)	0.70 (0.46–1.08)	0.106	0.75 (0.43–1.31)	0.315
Number of sexual partners†, median	**3 (2–5)**	**1.08 (1.01–1.16)**	**0.031**	1.06 (0.97–1.17)	0.176
1–2, n (%)	98 (28.8)	1.00 (Reference)		1.00 (Reference)	
3–5, n (%)	177 (52.1)	1.39 (0.84-2.30)	0.195	1.22 (0.67-2.22)	0.506
>5, n (%)	**65 (19.1)**	**1.96 (1.04-3.70)**	**0.038**	1.53 (0.70–3.38)	0.284
Contraception, n (%)					
No	225 (66.6)	1.00 (Reference)		1.00 (Reference)	
Yes	113 (33.4)	0.84 (0.54–1.33)	0.466	0.92 (0.54–1.85)	0.769
HIV status, n (%)					
Negative	309 (92.8)	1.00 (Reference)		1.00 (Reference)	
Positive	24 (7.2)	1.21 (0.53–2.77)	0.657	0.95 (0.36–2.53)	0.589
Age at first delivery (years), n (%)					
≤20	157 (47.7)	1.00 (Reference)		1.00 (Reference)	
≥21	172 (52.3)	0.70 (0.45–1.08)	0.102	0.60 (0.34–1.07)	0.085
Parity, n (%)					
Nulliparous	14 (4.1)	1.00 (Reference)		1.00 (Reference)	
1–4	**165 (48.5)**	**0.21 (0.06–0.79)**	**0.020**	0.26 (0.02-2.91)	0.274
>4	**161 (47.4)**	**0.23 (0.06–0.86)**	**0.029**	0.28 (0.02-3.22)	0.307
Transformation zone, n (%)					
TZ1	210 (65.8)	1.00 (Reference)		1.00 (Reference)	
TZ2	70 (22.0)	1.17 (0.68–2.02)	0.575	1.24 (0.67-2.26)	0.492
TZ3	**39 (12.2)**	**6.72 (2.84–15.93)**	**<0.0001**	**6.47 (2.59-16.21)**	**<0.0001**
HPV testing results, n (%)					
Other HPV (without co-infection)	264 (77.9)	1.00 (Reference)		1.00 (Reference)	
HPV-16/18/45	75 (22.1)	1.19 (0.70–1.98)	0.514	1.18 (0.64–2.17)	0.605
Cytology, n (%)					
High-grade+***	**29 (8.9)**	**2.47 (1.11–5.49)**	**0.027**	**3.37 (1.35–8.44)**	**0.009**

Abbreviations: 95% CI = 95% confidence interval; CIN2+ = cervical intraepithelial neoplasia grade 2 or worse.

*Data from the 340 participants may be missing for some variables.

†ORs for continuous variables indicate the change in odds for an increase of one standard deviation.

**Adjusted for age, marital status, age at first intercourse, number of lifetime sexual partners, age at first delivery, parity, HIV status, and type of transformation zone.

***High-grade lesions include ASC-H, HSIL, AIS, and cancer.

Bold values are statistically significant.

ABCD criteria were more likely to be correct in the presence of TZ type 3 (aOR = 6.47; 95% CI, 2.59–16.21; P<0·001) and high-grade lesions on cytology (aOR = 3.37; 95% CI, 1.35–8.44; P<0·009). Overall, a correct prediction of the ABCD criteria was not impacted by the multiple sociodemographic characteristics of the population in the multivariate analysis, apart

from women working as farmers who were less likely to have a correct prediction of ABCD criteria than employed women (OR 0.41, 95% CI 0.18-0.95).

Performance of ABCD and cytology for detection of high-grade cervical lesions (CIN2+ and CIN3+) is shown in **Table 3**.

Table 3: Diagnostic accuracy of ABCD criteria, cytology, and HPV for detection of CIN2+ and CIN3+

	CIN2+ (N=40, 11.8%)				HPV+ (N=358)
Variable	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Positivity rate % (95% CI)
ABCD criteria-positive	77.5 (61.3–88.2)	42.0 (36.5–47.7)	15.1 (10.8–20.8)	93.3 (87.6–96.5)	60.9 (55.6-65.9)
Cytology ASC-US+	80.0 (64.0–89.9)	87.5 (83.1–90.7)	47.1 (35.3–59.2)	96.9 (93.9–98.5)	21.6 (17.4-26.4
Cytology LSIL+	70.0 (53.5–82.6)	91.3 (87.4–94.1)	52.8 (39.1–66.2)	95.6 (92.4–97.5)	16.6 (12.9-21.1)
Cytology HSIL+	62.5 (46.1–76.5)	98.6 (96.3–99.5)	86.2 (67.0–95.1)	95.0 (91.8–97.0)	9.3 (6.6-13.0)
HPV-16/18/45+	37.5 (23.5–53.9)	79.9 (74.9–84.1)	20.9 (12.3–30.8)	90.5 (86.3–93.5)	23.3 (19.1-28.1)
	CIN3+ (N=27, 7.9%)				
	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	
ABCD criteria-positive	70.4 (49.6–85.2)	40.6 (35.2–46.1)	9.3 (6.0–14.1)	94.1 (88.5–97.0)	
Cytology ASC-US+	88.9 (68.9–96.7)	85.4 (80.9–89.0)	35.3 (24.7–47.6)	98.8 (96.4–99.7)	
Cytology LSIL+	81.5 (60.9–92.5)	89.7 (85.7–92.7)	41.5 (28.7–55.5)	98.2 (95.7–99.2)	
Cytology HSIL+	74.1 (53.2–87.8)	97.0 (94.3–98.4)	68.9 (49.0–83.7)	97.7 (95.2–98.9)	
HPV-16/18/45+	44.4 (26.2–64.3)	79.8 (75.0–83.9)	16.0 (9.2–26.4)	94.3 (90.8–96.6)	

Abbreviations: CIN2+ = cervical intraepithelial neoplasia grade 2 or worse; CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; Cytology ASC-US+ = ASC-US, LSIL, ASC-H, HSIL, AIS, and cancer; Cytology LSIL+ = LSIL, ASC-H, HSIL, AIS, and cancer; Cytology HSIL+ = ASC-H, HSIL, AIS, and cancer; HPV = human papilloma virus; HPV-16/18/45+ = HPV DNA test positive for HPV-16, HPV-18, and HPV-45; 95% CI = 95% confidence interval; PPV = positive predictive value; NPV = negative predictive value.

ABCD criteria for CIN2+ detection showed a sensitivity of 77·5% (95% CI, 61·3%–88·2%), specificity of 42·0% (95% CI, 36·5%–47·7%), PPV of 15·1% (95% CI, 10·8%–20·8%), and NPV of 93·3% (95% CI, 87·6%–96·5%). Cytology-classified HSIL+ for CIN2+ detection showed lower sensitivity of 62·5% (95% CI, 46·1%–76·5%), but higher specificity of 98·6% (95% CI, 96·3%–99·5%), PPV of 86·2% (95% CI, 67·0%–95·1%), and NPV of 95·0% (95% CI, 91·8%–97·0%). Meanwhile, cytology-classified ASC-US+ showed improved sensitivity of 80·0% (95% CI, 64·0%–89·9%) and specificity of 87·5% (95% CI, 83·1%–90·7%). Screening by HPV 16/18/45 genotyping alone had a much lower sensitivity of 37·5% (95% CI, 23·5–53·9) and a specificity of 79.9% (95% CI 74·9–84·1). When combining HPV 16/18/45 partial genotyping with VIA triage of other HPV types, sensitivity rose to 85·0% (95% CI, 70·2%-94·3%) and NPV to 94·4% (95% CI, 88·2%-97·9%), while specificity decreased to 33·7% (95% CI 28·3%-39·3%) and PPV to 14·6% (95% CI 10·3%-19·8%). ABCD criteria for CIN3+ lesion identification showed a sensitivity of 70·4% (95% CI, 49·6%–85·2%), specificity of 40·6% (95% CI, 35·2%–46·1%), PPV of 9·3% (95% CI, 6·0%–14·1%), and NPV of 94·1% (95% CI, 88·5%–97·0%).

DISCUSSION

The ABCD criteria were established to improve the performance of visual-based approaches for triage of HPV-positive women. Previous studies conducted in LMICs indicated that triage using traditional VIA criteria is not satisfactory for the detection of CIN2+ lesions, as the gain in specificity when adding VIA to HPV testing is obtained at the expense of an important loss in sensitivity.(6,7,10) The challenge for VIA screeners lies in interpreting the wide variability of cervical presentations, in populations where obstetric trauma to the cervix and history of infection are frequent, and in which CIN2+ may be difficult to identify.

The most important finding of this study is that the ABCD criteria appeared to be highly sensitive for detection of high-grade lesions in an HPV-positive population. We used both (i) a magnification technique with smartphone digital imaging that allows more detailed examination compared with naked eye alone and (ii) a lower VIA/D-VIA threshold positivity to optimize identification of lesions. The ABCD criteria provided improved VIA sensitivity for triage of HPV-positive women compared to most previous studies using a comparable methodology (histology as reference standard) (6,10,15,26,27) This can be explained by the fact that the IARC criteria require dense VIA changes before being considered positive, thus limiting their sensitivity, while a reduced positivity threshold can contribute to improved sensitivity for CIN2+ detection.(13,24)

The low specificity and PPV, leading to higher overtreatment rates, arise because we considered any whitening to be positive, meaning many benign conditions (metaplasia, inflammation or other benign cervical changes) could produce false-positive results for the ABCD criteria. Criterion C (VILI/D-VILI), though dependent on criteria A and D, may contribute to the high false positive rate by categorizing benign conditions as ABCD-positive through the identification of iodine-negative areas compatible with thin, transparent or patchy acetowhite lesions. Overall, 54·4% of normal histology results and 71·4% of CIN1 were considered ABCD criteria positive and consequently underwent unnecessary treatment. Thus, 85% (174 of 205) of women who screened positive were treated without CIN2+. However, when considering all women screened for CC, including HPV-negative, 174 were treated unnecessarily out of 1964 screened by Self-HPV, corresponding to an overall 8·9% overtreatment rate in the total population screened. Despite the low specificity, our 3T-Approach in a single visit may be acceptable in an LMIC context because it reduces cost and loss to follow-up, which are recognized barriers to effective cervical cancer screening.(11,28) Indeed, studies in Uganda(29) and South Africa(28) have shown loss to follow-up rates between 21% and 25% after the first visit, up to 50% at 24 months. Furthermore, treatment by thermal ablation is associated with very low risks of side effects and morbidity.(30) Therefore, treatment of a significant number of false-positive cases in this context may be

considered an acceptable strategy for effective control of CC in an LMIC setting and may contribute to reaching the target of the WHO's elimination initiative.(3,5) However, the use and integration of the ABCD criteria in the cervical cancer screening process warrants multidisciplinary discussion with involved stakeholders, taking into account the local context and resources, as well as regional HPV prevalence, prevalence of CIN2+ in HPV-positive participants, level of risk including HIV prevalence, availability of treatment modalities on site, and the possibility to offer further investigation when required. According to the context, the decision to refer has consequences for the patients and the health care system, requiring additional time and resources, and increasing the risk of loss to follow-up. Recognizing the limitations of the ABCD criteria with regard to PPV and overtreatment rates, other triaging strategies merit further investigation. The use of extended HPV genotyping (HPV 16, 18, 45, 31, 33, 35, 52 and/or 58) for the triaging of HPV-positive women is one alternative that should also be explored.

Compared to screening by HPV-16/18/45 genotyping without triage, the sensitivity of the ABCD criteria was much higher, at the cost of a lower specificity. PPV was also slightly lower with triage by ABCD criteria (15·1%) than with HPV partial genotyping (20·9%). One of the screening strategies currently recommended by the WHO is combined HPV 16/18/45 genotyping (treated immediately) and VIA triage of non-16/18/45 HPV genotypes.(3) In our

study population, this combined strategy resulted in an increased sensitivity of 85.0%, but even further decreased the specificity and PPV, which would therefore even further increase overtreatment rates. On the contrary, triage by cytology (using a threshold of ASC-US for a positive triage) improved both sensitivity (80·0%, 95% CI 64·0-89·9) and specificity (87·5, 95% CI 83·1-90·7) compared to the ABCD criteria. However, although this strategy may be adapted to higher-middle and high-income countries, the lack of trained cytotechnicians and well-equipped laboratories in low-income countries, the higher cost, and the inability to provide same-day treatment to patients positively triaged with cytology, render this triaging strategy unsuitable for low-resource settings. In comparison, the ABCD criteria require only basic equipment at a low cost, and allow initiation of therapy without delay. In our series, 86·7% of participants underwent the 3T-Approach in one day. ABCD criteria comprise a simple tool with binary results (positive or negative) that can alert healthcare professionals to the clinical features of CIN2+, and the use of “relaxed IARC criteria” may greatly decrease the risk of missing CIN2+ lesions. While digital imaging by smartphone may facilitate ABCD interpretation and enhance diagnostic performance, it may result in slightly prolonged examination time and may not be accessible in all settings.

Having a TZ3 was associated with a better prediction of ABCD criteria compared to TZ1 (**Table 2**), which is unexpected as VIA is generally considered inadequate for the evaluation

of TZ3 cervixes. This may be due to the use of B, C and D criteria in addition to acetowhiteness, enabling the detection of lesions extending to the ectocervix and bleeding in the absence of visible lesions. However, as A, B, C and D criteria were not assessed separately within this study sample, it is currently not possible to determine which criterion contributes most to a correct interpretation of VIA. A study is currently underway to assess each criterion individually for the detection of CIN2+. The lack of association between multiple socio-demographic variables and a correct prediction of the ACBD criteria (**Table 2**) supports the generalizability of these criteria to the overall population of women aged 30 to 49 years in West Cameroon. However, the limited sample size and the fact that the study was conducted in a single center, do not allow to extend these results to the overall female population, especially considering the differences in HPV prevalence in other regions. A further limitation is that the study was conducted in a single centre in a district hospital in West Cameroon with five health care providers administering all screening and treatment procedures.

It should be noted that two out of three cervical cancers were assessed as ABCD-negative on site by the frontline health care providers and did not receive immediate treatment. After reviewing the digital images of these two cases off-site, it was determined that criterion B

(bleeding) was present in both cases, which should have led to a positive ABCD result (**Supplement, Figure S1**).

Strengths of our study included the application of ABCD criteria upon VIA examination in real-life conditions with immediate treatment when necessary, therefore supporting the feasibility of a "screen-and-treat" strategy. Furthermore, because all HPV-positive women underwent biopsy and cervical brushing regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity for all diagnostic strategies assessed.

In conclusion, ABCD criteria can improve CIN2+ diagnosis in HPV-positive women and may provide a unique opportunity to improve cervical cancer screening programs in LMICs using a one-visit approach. This strategy may be particularly beneficial because the criteria are easily remembered and to use for healthcare providers.

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Contributors

PP, BK, and PV designed the study protocol, implemented the study, oversaw the data collection, analysed the data, and drafted and revised the paper. AW and RC conducted data analysis, interpreted the data, and revised the draft paper. BK, ET, and JF trained the study staff, assumed the quality control (supervision and mentorship), supported the data collection, interpreted the data, and revised the draft paper. JCT and ES analysed the pathological specimens, interpreted the data, and revised the draft paper.

Competing Interests

All authors declare that they have no competing interests.

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preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data access, analysis and responsibility

The principal investigator had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Data used in the study is available upon request to the first author.

Data sharing statement

Data are available upon reasonable request to the principal investigator of the study.

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Figure 1: ABCD criteria for VIA interpretation in HPV-positive women

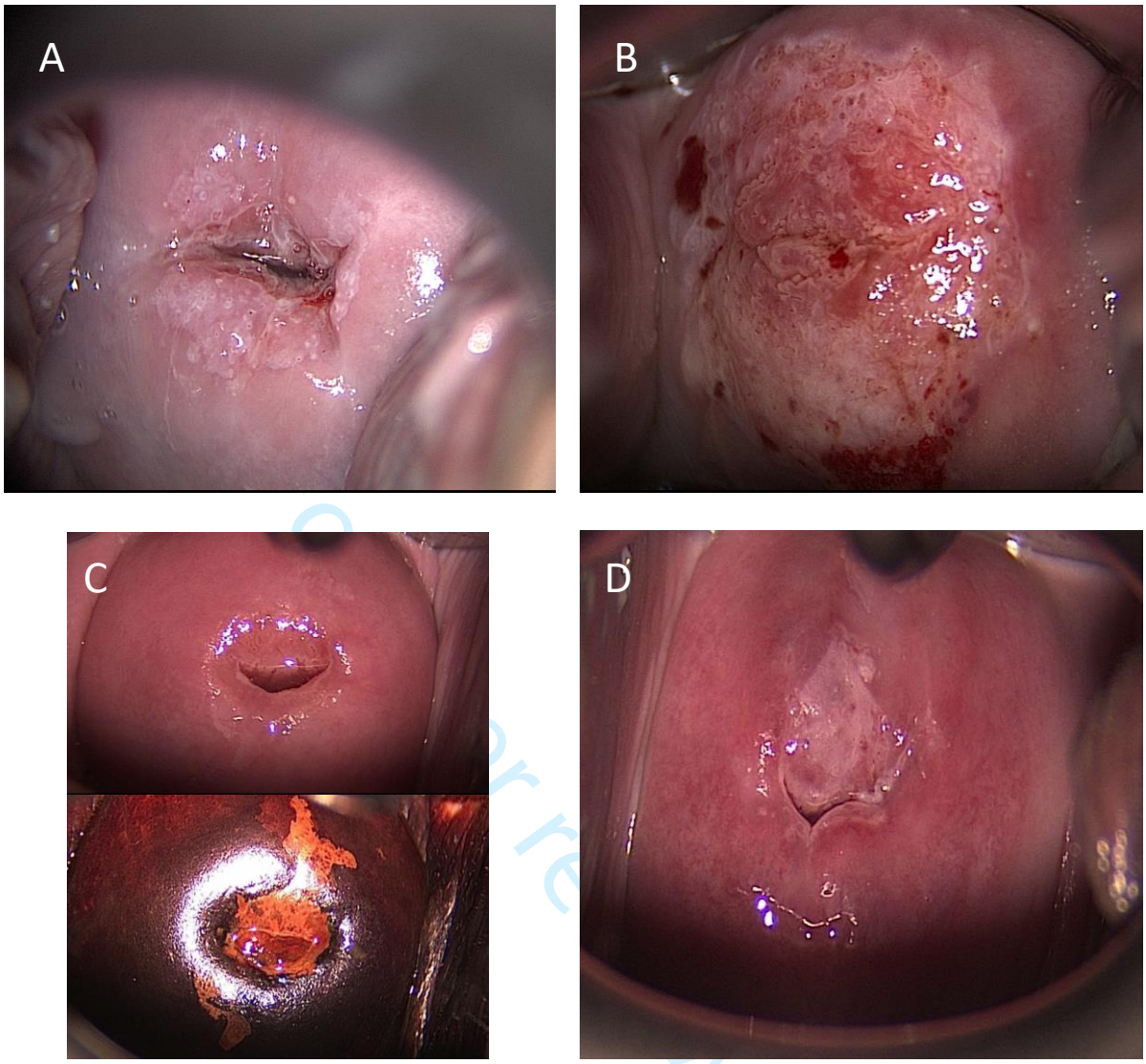
Criterion A – **A**cetowhite area touching the transformation zone (absent on the native view and apparent after acetic acid application) is considered positive.

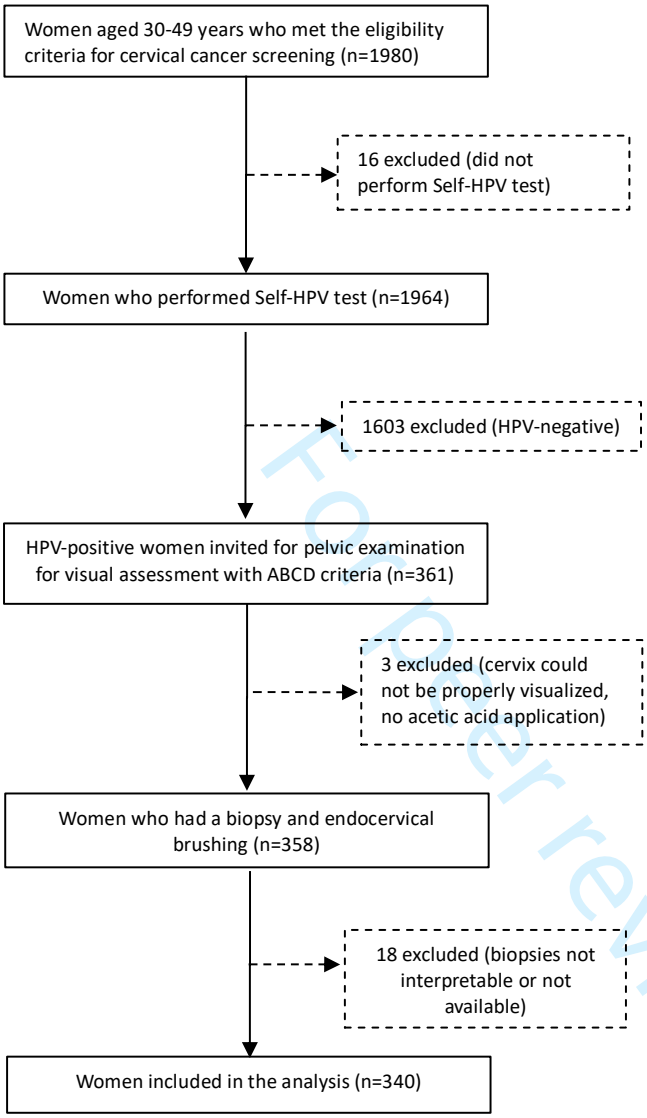
Criterion B – **B**leeding without touching or after lightly touching (with a swab or speculum) the cervix is considered positive.

Criterion C (optional) – **C**olouring with VILI contributes to confirmation or identification of a faint acetowhite lesion.

Criterion D – Diameter of >5 mm (about the size of a pencil eraser) in an acetowhite area is considered positive.

Figure 2: Flowchart of participants for the 3T-Approach in Cameroon



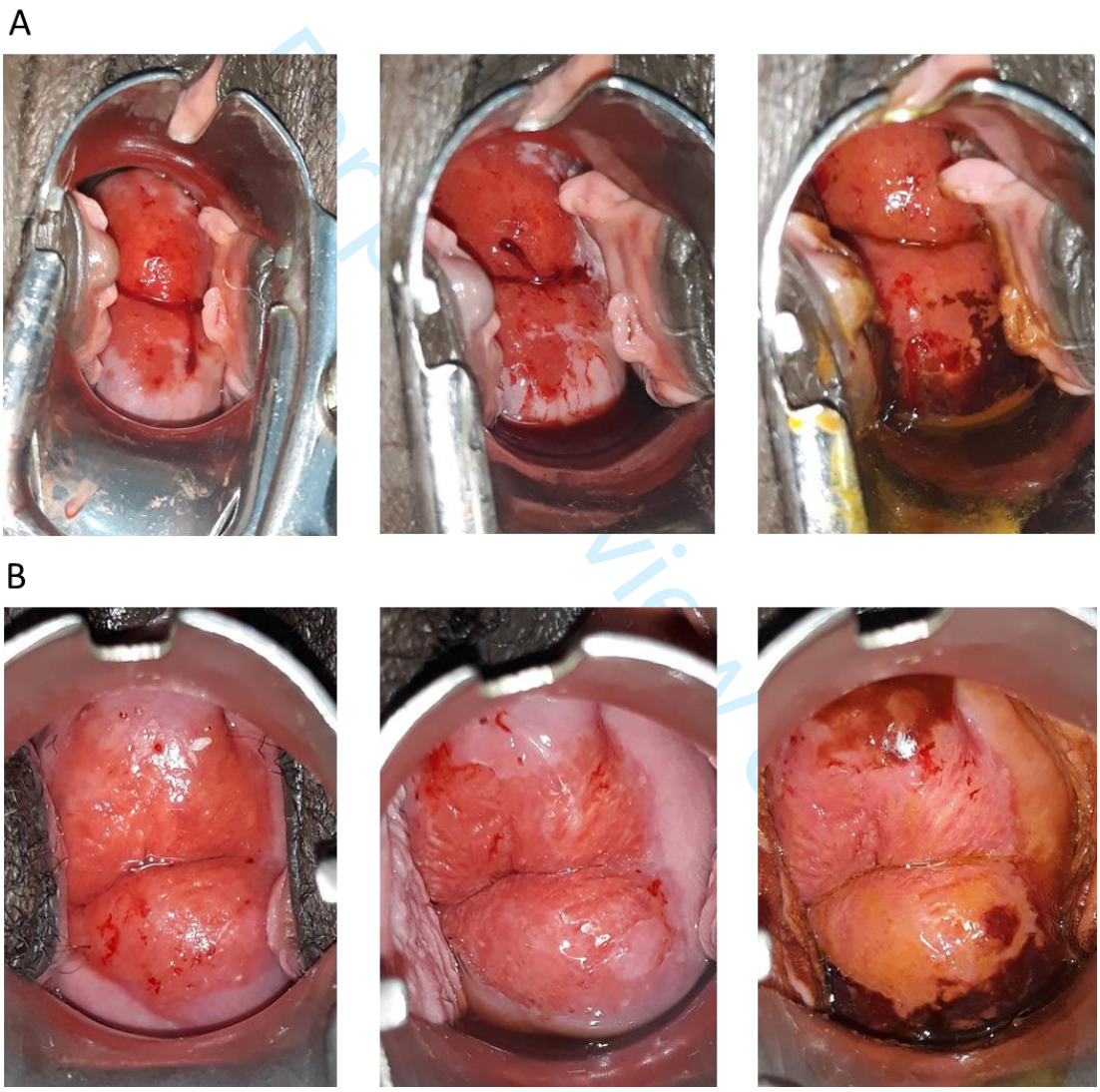


Supplementary Material

ABCD Criteria to Improve Visual Inspection with Acetic Acid (VIA) Triage in HPV-positive Women: a prospective analysis

Patrick Petignat, Bruno Kenfack, Ania Wisniak, Essia Saiji, Jean-Christophe Tille, Jovanny Tsuala Fouogue, Rosa Catarino, Evelyn Foguem Tincho and Pierre Vassilakos

Figure S1. Cases of cervical cancer not identified by ABCD criteria on site



A. Poorly differentiated carcinoma, positive for criterion B (bleeding); B. Invasive adenocarcinoma, positive for criterion B. From left to right, smartphone photos of (i) the native cervix, (ii) after application of acetic acid and (iii) after application of Lugol's iodine.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
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	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	5
Participants	**6**	Eligibility criteria	5
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	5
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	6 + figure 1
	10b	Reference standard, in sufficient detail to allow replication	7
	11	Rationale for choosing the reference standard (if alternatives exist)	na
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	7
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	6
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8
	15	How indeterminate index test or reference standard results were handled	8
	16	How missing data on the index test and reference standard were handled	8
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	na
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RESULTS			
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	21a	Distribution of severity of disease in those with the target condition	10-11
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	22	Time interval and any clinical interventions between index test and reference standard	na
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	10 (table 1)
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	12 (table 3)
	25	Any adverse events from performing the index test or the reference standard	10
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	15
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OTHER INFORMATION			
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STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.